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Evolutionary pathways of transmitted drug-resistant HIV-1

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Several large studies in Europe and the USA revealed that approximately 10% of all newly diagnosed patients harbour HIV-1 variants with at least one major resistance-associated mutation. In this review we discuss the underlying mechanisms that drive the evolution of drug-resistant viruses after transmission to the new host. In a comprehensive literature search 12 papers describing the evolution of 58 cases of transmitted resistant HIV-1 variants were found. Based on observations in the literature we propose three pathways describing the evolution of resistant HIV-1 after transmission to a new host. Firstly, reversion of the resistance mutation towards wild-type may rapidly occur when drug resistance mutations severely impact replicative capacity. Alternatively, a second pathway involves replacement of transmitted drug resistance mutations by atypical amino acids that also improve viral replication capacity. In the third evolutionary pathway the resistance mutations persist either because they do not significantly affect viral replication capacity or evolution is constrained by fixation through compensatory mutations. In the near future ultra-sensitive resistance tests may provide more insight into the presence of archived and minority variants and their clinical relevance. Meanwhile, clinical guidelines advise population sequence analysis of the baseline plasma sample to identify transmission of resistance. Given the limited sensitivity of this technique for minority populations and the delay between the moment of infection and time of analysis, knowledge of the described evolutionary mechanisms of transmitted drug resistance patterns is essential for clinical management and public health strategies.

Keywords: resistance, transmission, persistence, evolution

Introduction

Current guidelines supporting the earlier start of therapy and the recent introduction of new classes of potent and more tolerable antiretroviral drugs have reduced the number of HIV-infected individuals with detectable plasma HIV RNA in the developed world. Given the correlation between the level of HIV RNA in the individual and the risk of transmission,[1] it may be presumed that increased viral suppression at a population level will reduce the number of new infections.[2] Consequently, the individuals most likely to transmit HIV will be as follows: those unaware of their infection status; diagnosed patients with high CD4+ cell counts who are not yet eligible for therapy; or treatment-experienced patients with unsuppressed plasma viraemia caused by therapy failure. The latter group represents an obvious risk for transmission of drug-resistant HIV; several reports have indicated that 75%–80% of treated individuals with detectable plasma HIV RNA levels carry viruses with reduced susceptibility to one or more drugs.[3,4]

Surveillance studies have shown that approximately 10% of new HIV-1 infections involve drug-resistant strains, indicating that treated individuals are indeed involved in the spread of new infections. However, it has been shown that individuals infected *de novo* with drug-resistant viruses can also serve as a source of subsequent infections and thus contribute to the spread of drug-resistant HIV.[5–7]

The European HIV-1 surveillance programme Strategy to Control SPREAD of HIV Drug Resistance (SPREAD) is the largest ongoing prospective study investigating the transmission of drug-resistant HIV. In this programme, data and samples from recently diagnosed treatment-naive individuals are collected using a strategy enabling representative sampling among different transmission groups. The programme has revealed a stabilizing prevalence of viruses with drug resistance mutations of 8.4% in 2793 treatment-naive patients in 2002–05.[8] This level is comparable to that in the earlier retrospective European Combined Analysis of resistance Transmission over time of Chronically and acute infected HIV patients (CATCH) study (10.4%), which was conducted from 1996 to 2002.[9] Similar results have been reported in a large study in the USA. Between 1997 and 2001 over 1080 recently infected treatment-naive patients were enrolled, of whom 8.3% carried HIV-1 with resistance-associated mutations.[10] More recent data from patients diagnosed in 2006 in 11 surveillance areas in the USA showed a higher prevalence of 14.6%.[11] In studies conducted in specific risk groups, e.g. Caucasian homosexual men in large cities, even higher levels of transmitted resistance, of up to 25%, have been reported.[12–14] In resource-limited countries with a shorter history of antiretroviral

availability, infection with drug-resistant HIV-1 is less common.[15] Nevertheless, a recent report has shown that even in these settings transmission of resistance can be relatively frequent if exposure to therapy in the area is high.[16] Furthermore, high rates of mother-to-child transmission of drug resistance have been reported, especially in studies investigating the effect of single-dose nevirapine in resource-limited settings.[17] Recently, interventions with expanded drug regimens including short courses of nucleoside reverse transcriptase inhibitors (NRTIs) or replacement of single-dose nevirapine by a complete highly active antiretroviral therapy (HAART) regimen have shown decreased rates of transmission and resistance.[18,19]

Although information on follow-up of individuals with transmitted resistance is limited in the literature, most available cases show persistence of major drug resistance profiles for a long time.[20–29] Nonetheless, reversion of the transmitted drug resistance patterns in the plasma has been reported as well.[20–26,28,30,31] Furthermore, data from the CATCH study indicated a higher level of resistance in recently infected individuals compared with individuals with a longer or unknown duration of infection, suggesting that reversion of resistant viruses to drug-susceptible variants does occur rather frequently over time.[32] Additional evidence of viral evolution following infection comes from the frequent detection of atypical variants on drug resistance positions, mainly 215 in reverse transcriptase (RT), in newly diagnosed individuals.[30,33,34] These mutations, often representing molecular intermediates between drug-resistant mutants and wild-type, are rarely seen in treated individuals.

Thus, in the absence of drug selective pressure in the new host, transmitted drug-resistant viruses may revert to wild-type, evolve to other variants or persist. Insight into whether particular variants are likely to revert, evolve or persist has important implications for prevalence studies, public health and clinical management. This review discusses the current literature and will try to shed some light on the mechanisms that drive the evolution of resistant variants after transmission to a new host.

Methods

A comprehensive literature search using the primary search terms 'reversion' or 'persistence' combined with 'HIV', 'transmission' and 'resistance' on PubMed resulted in the selection of a number of papers describing the evolution of transmitted resistance in the new host in the absence of therapy.[22,24,28,29,31,35] Some additional papers regarding the evolution of drug-resistant HIV-1 after transmission that were found through citations were also included.[20,21,23,25–27,30]

Since it is only possible to distinguish between natural variation and transmitted resistance for mutations that are not present as variants in the natural quasispecies, this review focuses on primary mutations as listed in the International AIDS Society mutation table.[36] This list was chosen because it is used most often to characterize resistance-associated mutations. Secondary mutations may also be selected under drug-selective pressure, but may also appear as natural polymorphisms and therefore cannot be used as reliable indicators of exposure to antiretroviral drugs in a previous host. Recently, an updated consensus list of the WHO genotypic definition of transmitted drug resistance was published, which will be useful for future publications.[37]

In the literature, time after diagnosis or (presumed) infection is calculated in weeks or months. For convenience and transparency of the literature overview in Table 1, we calculated persistence over time in months. Described cases of superinfection[22] or a presumed recombination event[21] were excluded from this review. Several papers not only investigated mutations present in virus particles in plasma, but also analysed peripheral blood mononuclear cells (PBMCs) to study archived mutations[24,27] or performed clonal sequencing to look into the presence of viral subpopulations.[24,28,31] These data are included in the Results section, but not in Table 1.

Results

A total of 58 cases infected with drug-resistant HIV-1 from 12 papers were included. An overview of these cases and the evolution of the particular mutational patterns over time are displayed in Table 1. These papers focused either on the RT domain or on both RT and protease (PR).

One of the first papers describing the evolution of transmitted resistance mutations in RT described patients included in a Swiss cohort.[30] Yerly *et al.*[30] reported four seroconverters who were infected with viruses harbouring mutations in RT associated with resistance to thymidine analogues (TAMs). Over time, partial reversion of resistance was observed in all cases with persistence of the M41L only. At position 215 the resistant variants phenylalanine and tyrosine (F and Y, respectively) were replaced in plasma by variants other than wild-type. The Amsterdam cohort reported similar findings in eight patients infected with a virus containing TAMs.[20]

Several other groups have reported on more extensive transmitted resistance.[21,22,25,26,29,31] In general, in the absence of treatment, the RT mutation M184V became undetectable by population sequencing and 215 mutations are replaced by other variants. Within the extensive PR profiles, individual mutations occasionally reverted over time, but the majority of mutations persisted.

In the San Diego cohort, multiple cases of transmitted non-nucleoside reverse transcriptase inhibitor (NNRTI)-related resistance were followed during their treatment-naive period.[28] The NNRTI resistance mutation K103N was seen in 11 men who have sex with men and reverted to wild-type in only two cases; incomplete reversion was observed in two other patients after 38 and 212 weeks. Other NNRTI resistance mutations reverted only partially (Y181C and P225H) or persisted (Y188L) during follow-up. Some resistance mutations appeared in mixture during follow-up; these mutations might have been present at a level near the detection threshold of the assay.

Limited information is available on the evolution of resistance in other genes. Besides the PR gene itself, the PR substrate Gag also plays an important role in the development of resistance against PR inhibitors (PIs), but is not included in this review.[38] Polymorphisms and resistance-related mutations in Gag are not well defined yet, making it difficult to distinguish between transmitted drug resistance and natural variation. Of interest, a higher prevalence of specific Gag resistance mutations has been described in patients infected with viruses also harbouring PI mutations compared with patients infected with wild-type viruses.[39] Unfortunately, no follow-up data on the evolution of these profiles have been reported. Also, for newer drug classes such as entry and integrase inhibitors, more information on the natural variation in their target genes is necessary to enable identification of indicators of transmitted resistance to new compounds. Two groups reported transmission of viruses with resistance mutations to the fusion inhibitor enfuvirtide.[40,41] However,

Table 1. Evolution of major transmitted resistance mutations in plasma viral RNA

Patient ID	Months after infection or diagnosis	Resistance mutations		Pathway			First author (reference)
		RT	PR	reversion	atypical variants	persistence	
Profiles containing only NRTI-related mutations							
H	**0, 28**	**M41L**	—			M41L	Pao[26]
I	**0**	**A62V**	—				Pao[26]
	2	—		A62V → WT			
B	**0, 15**	**T69N**	—			T69N	Pao[26]
G	**0, 32**	**T69N**	—			T69N	Pao[26]
M	**0, 16**	**T69N**	—			T69N	Pao[26]
8	**0**	**K70R**	**ND**				de Ronde[20]
	36	—		K70R → WT			
B	**0**	**T215F**	**ND**				Yerly[30]
	6	**T215L**			T215F → L		
	12	T215L/F			T215L → L/F		
E	**0, 6**	**T215Y**	**ND**				Yerly[30]
	12, 16, 18	T215C			T215Y → C		
3	**0**	**T215Y**	**ND**				de Ronde[20]
	6	T215Y/S		T215Y → Y/S			
	12	T215S		T215Y/S → S			
K	**0, 11**	**T215D**	—			T215D	Pao[26]
N	**0, 13**	**T215D**	—			T215D	Pao[26]
J	**0**	**K219Q**					Pao[26]
	9, 22, 36	—		K219Q → WT			
35	**0**	**M41L, T215Y**	—				Ghosn[27]
	6	M41L, T215Y/C			T215Y → Y/C		
	12	M41L, T215C			T215Y/C → C		
	24	M41L, T215C				M41L	
E	**0**	**M41L, T215Y**	—				Pao[26]
	21	M41L, T215C			T215Y → C		
	33	M41L, T215C				M41L	
G	**0**	**M41L, T215Y**	**ND**				Yerly[30]
	42	M41L, T215D			T215Y → D	M41L	
1	**0**	**M41L, T215Y**	**ND**				de Ronde[20]
	6, 12	M41L, T215Y/N		T215Y → Y/N			
	18	M41L, T215N		T215Y/N → N			
	24, 30, 36	M41L, T215N/D		T215N → N	T215N → D	M41L	
2	**0**	**M41L, T215Y**	**ND**				de Ronde[20]
	6	M41L, T215Y/N		T215Y → Y/N			
	12	M41L, T215N/D		T215Y/N → N	T215Y/N → D	M41L	
4	**0**	**M41L, T215Y**	**ND**				de Ronde[20]
	6	M41L, T215D			T215Y → D		

Continued

Table 1. *Continued*

Patient ID	Months after infection or diagnosis	Resistance mutations		Pathway			First author (reference)
		RT	PR	reversion	atypical variants	persistence	
	12, 18, 24	M41L, T215D/S		T215D→S	T215D→D		
	30	M41L, T215D/S				M41L	
7	**0, 6**	**M41L, T215D**	**ND**			M41L, T215D	de Ronde[20]
C	**0, 7**	**M41L, T215L**				M41L, T215L	Pao[26]
34	**0, 24**	**M41L, L210W, T215C**				M41L, L210W, T215C	Ghosn[27]
5	**0**	**D67N, K70R, T215F**	**ND**				de Ronde[20]
	6	D67N/D, K70R, T215S/L		D67N→N/D, T215F→S	T215F→L	K70R	
6	**0, 6**	**D67N, K70R, K219Q**	**ND**			D67N, K70R, K219Q	de Ronde[20]
B	**0**	**D67N/D, K70K/R, K219G**	—				Barbour[25]
	6	K70K/R, K219G		D67D/N→WT			
	15	K70R, K219G				K70R, K219G	
F	**0**	**D67N, K70R, T215F, K219Q**	**ND**				Yerly[30]
	46, 58	—		D67N, K70R, T215F, K219Q→WT			
Profiles containing only NNRTI-related mutations							
36	**0, 24**	**K103N**	—			K103N	Ghosn[27]
01–0125	**3, 4**	**K103N**	—			K103N	Little[28]
01–0143	**1.5, 2.5, 4, 5, 6, 6.5**	**K103N**	—			K103N	Little[28]
01–0566	**3, 6, 9, 13, 16.5, 22, 25.5, 27, 29.5, 34**	**K103N**	—			K103N	Little[28]
01–0183	**3, 4, 5**	**K103N**	—			K103N	Little[28]
01–0503	**1, 1.5, 3.5, 5.5, 8.5, 10.5, 13.5**	**K103N**	—			K103N	Little[28]
01–0180	**3, 4, 5, 6**	**K103N**	—				Little[28]
	6.5, 7.5, 8.5, 9.5, 10.5, 13.5, 17, 19.5, 23.5, 23.5, 27.5, 31	K103K/N		K103N→K103K/N			
	34, 37, 39.5, 42.5	—		K103K/N→WT			
01–0512	**2, 2.5, 5, 6.5, 8.5, 11, 15, 18, 21, 25.5, 28.5, 36**	**K103N**	—				Little[28]
	31, 34, 38.5, 41,5	K103N, P225P/H			+P225P/H		
	49	K103K/N		P225P/H→WT, K103N→K/N			
A	**0**	**Y181C**					Pao[26]
	25	—		Y181C→WT			
2	**0, 5**	**G190A**	—			G190A	Chan[23]
Patient F	**0**	**K103N, V108I/V**					Pao[26]

	23	K103N		V108I → WT		K103N	
01–0182	**3, 4.5, 5, 7.5**	**K103N, Y181C**	—				Little[28]
	8.5, 10.5	K103K/N, Y181Y/C, G190G/A		K103N → K/N, Y181C → Y/C	+G190A/G		
Profiles containing both NRTI- and NNRTI-related mutations							
01–0559	**1, 3.5, 6, 10, 11.5, 14, 18, 22, 25.5, 35**	**K103N, P225H**	—				Little[28]
	30.5	K103N, K219K/R, P225H			+K219K/R		
	39.5	K103N, P225P/H		K219K/R → WT, P225H → P/H		K103N	
01–0629	**1.5**	**D67N, K103K/N, M184M/V, T215S, K219E**	—				Little[28]
	5, 7.5, 9.5, 13, 16.5, 21, 26, 29, 31.5, 37, 40	D67N, T215S, K219E		M184M/V, K103K/N → WT		D67N, T215S, K219E	
Profile containing only PI-related mutation							
2	**0, 10**		**M46I**			M46I	Polilli[29]
Profiles containing both RT- and PI-related mutations							
33	**0**	**M41L, T215Y**	**I84V, L90M**				Ghosn[27]
	6	M41L, T215Y	I84V, L90M				
	12	M41L, T215Y/C	I84V, L90M		T215Y → Y/C		
	24	M41L, T215C	I84V, L90M		T215Y/C → C		
	36	M41L, T215C	I84V, L90M				
	48	M41L, T215C	I84V, L90M			M41L, I84V, L90M	
C	**0, 9**	**M41L, M184V, T215Y**	**M46I, I54M, V82A, L90M**			M41L, M184V, T215Y, M46I, I54M, V82A, L90M	Barbour[25]
1	**0, 2**	**M41L, L210W, T215C**	**V82A, L90M**				Chan[23]
	5	M41L, E44D, M184V, L210W, T215C	V82A, L90M		+E44D, M184V		
	9	M41L, E44D/E, M184M/V, L210W, T215C	V82A, L90M	E44D → D/E			
	12	M41L, E44D, M184M/V, L210W, T215C	V82A, L90M	E44D/E → D			
	14	M41L, E44D/E, L210W, T215C	V82A, L90M	M184V → WT, E44D → D/E			
	16	M41L, E44D, L210W, T215C	V82A, L90M	E44D/E → D			
	19	M41L, E44D, L210W, T215C	V82A, L90M				
	22	M41L, E44D/E, L210W, T215C	V82A, L90M	E44D → D/E			
	25	M41L, L210W, T215C	V82A, L90M				

Continued

Table 1. *Continued*

Patient ID	Months after infection or diagnosis	Resistance mutations		Pathway			First author (reference)
		RT	PR	reversion	atypical variants	persistence	
	26	M41L, E44D, L210W, T215C	V82A, L90M				
	29	M41L, E44D/E, L210W, T215C	V82A, L90M	E44D/E → D			
	32	M41L, E44D/E, L210W, T215S	V82A, L90M	T215C → S		M41L, L210W, V82A, L90M	
D	**0, 12**	**M41L, 210W, T215C**	**D30N, M46L**			M41L, L210W, T215C, D30N, M46L	Barbour[25]
01–0449	**3, 4, 6, 8, 10, 12, 15.5, 23**	**M41L, D67N, K70R, T215Y, K219Q**	**D30N, M46I, I84V, L90M**				Little[28]
	14, 20, 31, 43.5	M41L, D67N, K70R/K, T215Y, K219Q	D30N, M46I, I84V, L90M				
	27, 35, 37, 46	M41L, D67N, T215Y, K219Q	D30N, M46I, I84V, L90M	K70R → WT			
	49, 52	M41L, D67N, T215Y/C, K219Q	D30N, M46I, I84V, L90M		T215Y → Y/C	M41L, D67N, K219Q, D30N, M46I, I84V, L90M	
01–0575	**3, 6, 8.5**	**M41L, L74V, M184V, L210W, T215Y**	**M46I, V82A**				Little[28]
	11.5	M41L, L74V, M184M/V, L210W, T215Y	M46I, V82A				
	15, 18.5	M41L, L74V, L210W, T215Y, K219K/R	M46I, V82A	M184V → WT	+K219R/K		
	22.5	M41L, L74V, L210W, T215C/S	M46I, V82A	K219R/K → WT, T215Y → S	T215Y → C		
	25.5, 28	M41L, L74V, L210W, T215C/S	M46I, V82A/V	V82A → A/V			
	30.5	M41L, L74V, Y181Y/C, L210W, T215C/S	M46I, V82A	V82A/V → A	+Y181Y/C		
	33, 35	M41L, L74V, Y181Y/C, L210W, T215C/S	M46I, V82A/V	V82A → A/V			
	39	M41L, L74V, Y181Y/C, L210W, T215S	M46I, V82A/V	T215C/S → S		M41L, L74V, Y181Y/C, L210W, M46I	
PHI 3	**3, 4, 6.5**	**K103N**	**I54V, V82T, I84V, L90M**				Brenner[21]
	7.5, 8.5	K103N		I84V → WT		K103N, I54L, V82T, L90M	
PHI-3	**2, 3, 5, 8, 9, 39**	**K103N**	**I54V, V82T, I84V, L90M**			K103N, I54V, V82T, I84V, L90M	Brenner[22]
PHI 2	**2**	**M41L, K103N, M184V, T215Y**	**G48V, V82A, L90M**				Brenner[21]
	3	M41L, K103N, M184V, T215Y	G48V/G, V82A, L90M				

	5.5, 6.5	M41L, K103N, M184V, T215Y	V82A, L90M	G48V→WT			
	7.5	M41L, K103N, M184V/M, T215Y	V82A, L90M	M184V→V/M			
	8.5	M41L, K103N, T215Y	V82A, L90M	M184V/M→WT			
	11	M41L, K103N, T215C	V82A, L90M		T215Y→C	M41L, K103N, V82A, L90M	
PHI-2	**2, 3, 6, 7, 8, 9**	**M41L, K103N, M184V, T215Y**	**G48V, V82A, L90M**				Brenner[22]
	12	M41L, K103N, T215Y	V82A, L90M	M184V, G48V→WT		M41L, K103N, T215Y, V82A, L90M	
L	0, 18	T69N, Y188L, K219Q	I54L, I84V, L90M			T69N, Y188L, K219Q, I54L, I84V, L90M	Pao[26]
32	**0, 6**	**M41L, K103N, L210W, T215Y**	**L90M**				Ghosn[27]
	12	M41L, K103N, L210W, T215Y/C	L90M		T215Y→Y/C		
	24	M41L, K103N, L210W, T215C	L90M		T215Y/C→C	M41L, K103N, L210W, L90M	
01–0507	**1.5, 2.5, 3.5, 5.5**	**M41L, D67N, K103N, T215Y**	**I84V, L90M**				**Little**[28]
	7, 8	M41M/L, D67N, K103N, T215C/Y	I84V, L90M				
	9.5	M41L, D67N, K103N, T215C/Y	I84V, L90M				
	14, 16	M41L/M, D67D/N, K103N, T215C/D/G/Y	I84V, L90M	M41L→L/M, D67N→D/N	T215Y→C/D/G/Y	K103N, I84V, L90M	
	15	M41L, K103N, T215C	M46L, I54V, V82A, L90M		T215Y/C→C	M41L, K103N, M46L, I54V, V82A, L90M	
B	0	**D67N, T69D, K103N, Y181C, T215L**	**M46I, L90M**				**Delaugerre**[24]
	24, 35	D67N, T69D, K103N, Y181C, T215L	L90M	M46I→WT		D67N, T69D, K103N, Y181C, T215L, L90M	
01–0483	**3, 3.5**	**M41L, V75I, Y188L, T215L, K219E**	**D30N**			M41L, V75I, Y188L, T215L, K219E, D30N	**Little**[28]
A	**0**	**M41L, K103N, M184V, L210W, T215Y**	**M46L, I54V, V82A, L90M**				**Barbour**[25]
	6	M41L, K103N, M184V/M, L210W, T215Y	M46L, I54V, V82A, L90M				
	10	M41L, K103N, L210W, T215Y	M46L, I54V, V82A, L90M	M184V→WT			
	12	M41L, K103N, L210W, T215Y/C	M46L, I54V, V82A, L90M		T215Y→Y/C		
	15	M41L, K103N, T215C	M46L, I54V, V82A, L90M		T215Y/C→C	M41L, K103N, M46L, I54V, V82A, L90M	

Continued

Table 1. *Continued*

Patient ID	Months after infection or diagnosis	Resistance mutations		Pathway			First author (reference)
		RT	PR	reversion	atypical variants	persistence	
D	**0, 1, 9, 17**	**M41L, E44D, K103N, L210W, T215Y, K219R**	**I54V, V82A, L90M**			M41L, E44D, K103N, L210W, T215Y, K219R, I54V, V82A, L90M	**Pao**[26]
index patient	**2**	**E44D, D67N, L74V, Y181V, G190S, T215D**	**V82T**				**Neifer**[31]
	19	E44D/E, D67N/D, L74V, Y181V, G190S, T215D	V82T	E44D→E/D, D67N→N/D			
	28	L74V/L, Y181V, G190S, T215D	V82T	E44E/D, D67N→WT L74V→V/L, V82T→A	Y181V, G190S, T215D,		

WT, wild-type; ND, not determined (sequencing of protease was not performed in all papers).
Data were ordered based on mutational profile and divided based on related drug class (NRTI, NNRTI and PI) and complexity of profile.
Only results obtained by population sequencing were included in the table.
The first detected profile is in bold font and evolutionary patterns are in normal font.
If multiple evolutionary pathways are observed, the mutations are depicted in all relevant columns.

long-term persistence or reversion after transmission of variants resistant to this inhibitor has not yet been documented. Only one case of transmitted resistance against integrase inhibitors has been described so far. In this case the integrase resistance mutations G140S and Q148H persisted for 48 weeks.[42] It would be of interest to investigate the evolution of transmitted resistance against integrase inhibitors in more depth.

Evolutionary pathways of transmitted drug resistance

After the complete removal of drug selection pressure in treated individuals, a rapid reappearance of wild-type HIV that persisted in the cellular compartment has been observed.[43] After transmission of drug-resistant HIV to a new host, such a rapid shift is unlikely given that wild-type virus is rarely co-transmitted.[44] Therefore, when only drug-resistant variants are present in the new host, a novel starting point for viral evolution is created; there is no 'memory' of the original wild-type in the quasispecies. Nucleotide changes in the quasispecies are modulated by chance events and will be selected if they have a beneficial effect on viral fitness. Transmitted drug-resistant variants may persist or fade away from detection in the plasma depending on their relative fitness in the new environment.

As summarized in Table 1, particular transmitted drug resistance mutations or patterns seem to persist in the plasma quasispecies while others are lost over time. We identified three possible evolutionary pathways of viral evolution after transmission, caused by different underlying mechanisms.

Pathway I: evolution to wild-type (reversion of transmitted drug resistance)

It is widely accepted that most major drug resistance mutations in RT and PR lower the replicative capacity (RC) of HIV.[45–47] If there is no beneficial effect of the resistance mutations after transmission to a new host, reversion to wild-type may be observed. This pathway is graphically depicted in Figure 1.

A striking example from the reviewed literature as summarized in Table 1 is the NRTI resistance-associated mutation M184V,

Figure 1. Evolution to wild-type. After transmission, the RC (*y*-axis) of the transmitted drug-resistant variant (TR) is lower than that of wild-type (WT). Due to complete (1) or incomplete (2) reversion of the drug resistance mutation, the RC is restored or improved.

which was replaced by wild-type in the plasma in six out of seven cases within 16 months (median 9.5 months; range 5–15 months). This mutation persisted in only one case, together with a combination of RT mutations (M41L, M184V, T215Y and K219G in RT) in a virus also harbouring multiple PI mutations.[25] The M184V significantly affects the RC of HIV-1.[48] Since the difference between wild-type and the drug-resistant variant is only one nucleotide change ($ATG_{wild\text{-}type} \rightarrow GTG_{var}$), a virus containing only the M184V mutation can rapidly revert to more replication-competent wild-type virus.

Incomplete reversion can be observed after transmission of resistance patterns that require more than one mutation for full reversion to wild-type. This is commonly observed at particular amino acid positions where two nucleotide changes are necessary to revert from the mutant form to wild-type. Incomplete reversion may result in intermediates. Such intermediates or revertants are commonly observed at codon 215 in RT and codon 82 in PR. The T215Y and T215F mutations are selected by thymidine analogues and cause (cross) resistance to all NRTIs.[20,33] Both changes have a considerable impact on RC.[49] It appears that the intermediate variants T215S, T215N and T215I have considerably higher RC than T215Y and T215F, almost approaching the RC of wild-type.[20] As a result, these intermediate variants tend to persist, as evident in Table 1. Of the 16 patients infected with a viral variant containing either the T215Y or T215F mutation, five were replaced with intermediates that persisted in four cases.

Pathway II: evolution towards atypical variants

After transmission of drug-resistant HIV to a new host, a novel amino acid may be selected that is neither the wild-type amino acid nor an intermediate towards wild-type. In general, these atypical variants confer a higher RC than the originally transmitted drug-resistant amino acid (Figure 2). On some occasions, these atypical variants represent amino acids that have been encountered in the untreated population and have been described as natural polymorphisms.

This phenomenon is often observed at position 215 in RT. At this position not only revertants are observed (as described above), but also variants that are not obvious intermediates between resistant variants and wild-type. Like revertants, these atypical variants have an increased RC and increased susceptibility to thymidine analogues compared with the originally transmitted drug-resistant variant. Figure 3 provides an overview of all the identified variations and their possible evolutionary pathways at position 215.

Selection of a particular pathway may depend on the genetic variation in the drug-resistant variant. For instance, Garcia-Lerma *et al.*[33] found that if the TAM M41L or L210W was present, the atypical variants T215D and T215C were observed more frequently than the intermediate T215S. However, when changes at codon 215 were present in isolation T215S was selected more often. We observed a similar trend in the reviewed literature for T215D and C, but not for T215S, possibly because this mutation was selected in only four cases.

Interestingly, data from the World-wide Analysis of resistance Transmission over time of Chronically and acute infected HIV patients (WATCH) study indicate that novel variants at resistance positions are more often observed in newly diagnosed patients infected with other drug resistance changes compared with patients infected with a wild-type background.[16] This observation provides further evidence that they develop from resistant variants rather than from natural variation of wild-type.

Pathway III: persistence of transmitted drug resistance

Three mechanisms can explain the persistence of resistant variants in the new untreated host.

Persistence because of a minimal reduction in RC

If the RC of transmitted drug-resistant variants is almost equal to that of wild-type, the replicative advantage of wild-type is limited and mutations may persist for a considerable time (Figure 4). For example, NNRTI resistance mutations, particularly K103N, have only a moderate effect on RC.[50] In the reports included in this review, transmission of K103N-containing variants was observed 21 times and persisted completely in 17 out of 21 patients for a median duration of 16 months (Table 1). Most other NNRTI resistance mutations also seem to be able to persist. In addition, the NRTI-associated mutations L210W and K219R/G/Q/E, which have

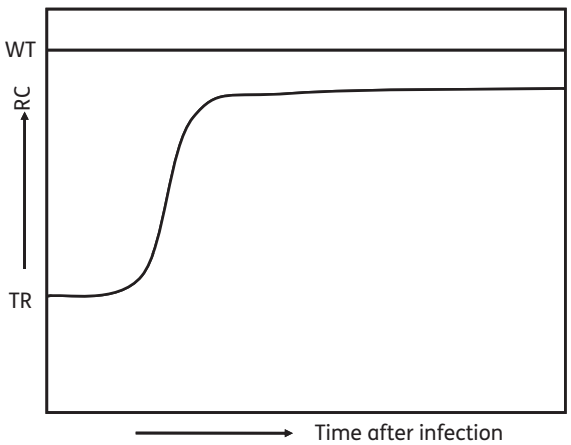

Figure 2. Selection of atypical mutations. After transmission of a drug-resistant HIV variant, atypical amino acids that are neither wild-type nor intermediates may be selected, leading to improved RC.

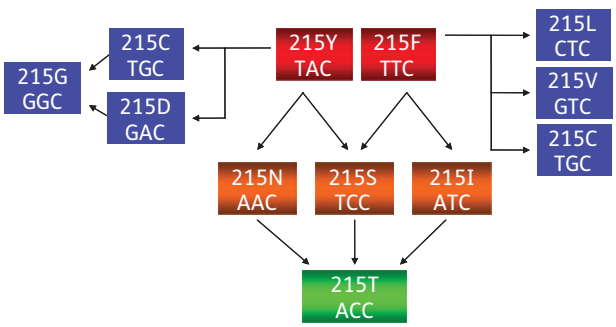

Figure 3. Evolution of transmitted variants at position 215 of RT. Variants T215Y and T215F (depicted in red) cause resistance to NRTIs. 215S/N/I (orange) are intermediates between resistant and wild-type, and 215C/D/L/V/C/G (blue) are atypical variants. The wild-type amino acid at position 215 is threonine (T) (depicted in green).

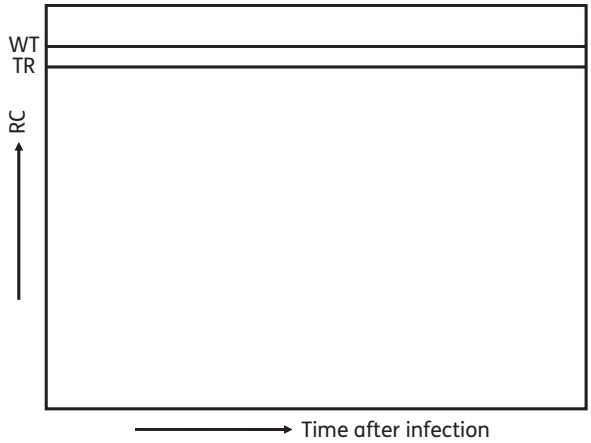

Figure 4. Persistence because of a minimal reduction in RC. If the RC of the resistant variant (almost) equals the RC of wild-type, persistence may occur for a considerable time.

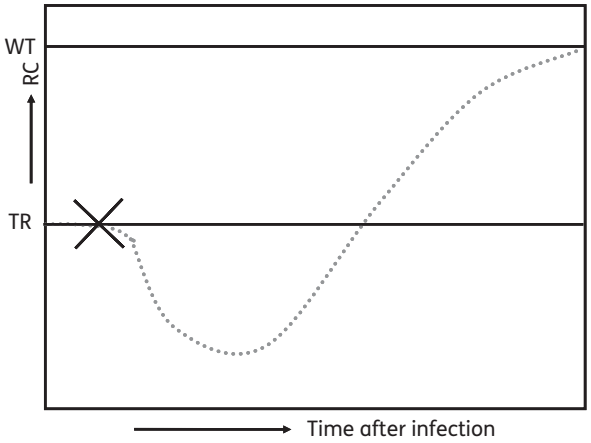

Figure 5. Reversion is blocked by compensatory fixation. Due to compensatory mutations, multiple mutations are required for full reversion. The first mutation would decrease the RC, so reversion is blocked.

also been reported to exert little effect on RC, also persisted in most cases.[49]

Persistence because reversion is prohibited by compensatory fixation

Interestingly, there are several examples of resistant viruses with an RC significantly lower than that of wild-type persisting after transmission to a new host. We proposed a mechanism called compensatory fixation to explain the persistence of combinations of PI-resistant mutations in patients interrupting their PI therapy.[51,52]

In the treated source, compensatory mutations may appear after the initial selection of drug resistance mutations that lower the RC. After transmission to a new host, evolution may be expected to occur in a stepwise manner. However, if all possible nucleotide changes would initially decrease the RC, reversion to wild-type will be blocked (Figure 5).

We propose that this mechanism may help to explain the persistence of some transmitted drug resistance genotypes. Table 1 shows that variants with PR-associated resistance mutations generally tend to persist. Persisting profiles are often characterized by the presence of several major PR resistance mutations, as well as secondary mutations that may compensate at least partially for the loss of RC induced by the major mutations.

Moreover, we have preliminary data that suggest that compensatory fixation also explains the persistence of a mutation in RT. The M41L mutation, which has a significant negative effect on RC, persisted for up to 7 years in combination with potential compensatory mutations in a large cluster of untreated patients.[53] Similarly in our literature review the M41L mutation persisted in all but one patient.

Selection of additional compensatory mutations that offset the effects of transmitted drug-resistant variants on RC is another possibility leading to persistence due to compensatory fixation. We have shown *in vitro* that, in the absence of drug selection pressure, PI-resistant viruses can select compensatory mutations that increase viral replication.[52] However, we did not find evidence for this evolutionary pathway in the reviewed literature; most likely because the papers focus on known primary drug resistance mutations.

Implications of transmitted drug resistance

As stated before, viruses with one or more drug resistance mutations generally have a reduced RC compared with wild-type virus in the absence of antiretroviral selection pressure. It has been suggested that, due to the diminished RC, increases in viral load and reduction of CD4+ cells may be slower in patients infected with drug-resistant HIV compared with patients infected with wild-type virus.[54,55] Other groups did not confirm such observations.[13,56,57]

Similarly, it may be expected that the disappearance of resistance mutations from the dominant replicating plasma population will increase the overall RC and viral fitness. Several reports indeed mention that replacement of T215Y or T215F by another variant resulted in a significant increase in HIV-1 RNA plasma levels.[20,22] Disappearance of other drug resistance mutations has also been reported to coincide with increased HIV RNA plasma levels.[21,23,35] A decline in CD4+ levels after reversion has also been reported, suggesting that wild-type virus has a stronger negative effect on CD4+ turnover.[23]

Transmission of drug-resistant variants may also influence clinical outcome. In the EuroSIDA cohort virological failure was retrospectively analysed in treatment-naive individuals who were infected with drug-resistant or drug-susceptible HIV-1.[58] Only a small and insignificant difference was found in terms of viral suppression. Unfortunately, it is not clear whether treatment was guided by genotypic and/or phenotypic analysis for optimal drug selection. Similar results were described by Oette *et al.*[59] after analysing a selection of 269 patients in whom therapy was selected after baseline genotypic and phenotypic analysis. They did not observe any significant increase in virological failure in individuals infected with drug-resistant virus over those infected with fully susceptible virus. However, the potential adverse consequences of an infection with drug-resistant HIV are obvious, in that the efficacy of future treatment will be

compromised if therapy is not modified. It has been shown that transmission of drug-resistant HIV can result in delayed viral suppression and/or accelerated virological failure as a result of the use of suboptimal therapy not guided by baseline resistance analysis. Recently, a large collaborative project, European collaboration of HIV observational cohorts (EuroCoord) and the European Collaborative HIV and Anti-HIV Drug Resistance Network (CHAIN), looked into the effect of transmitted drug resistance on virological treatment outcome. Over 10000 patients were included in this analysis. In this study, patients infected with a resistant virus who received an NNRTI-based regimen had a higher risk of virological failure compared with patients infected with wild-type virus, even when this regimen was adapted to the presence of resistance mutations. This effect was not observed for patients receiving a therapy containing boosted PIs, probably due to the higher genetic barrier of these regimens.[60] These studies describe pooled analyses of different resistance profiles related to the outcome of various regimens. Currently the generalizability of these data is uncertain. For instance, it is not yet known whether the frequently observed transmission of single TAMs, which confer resistance to early NRTIs such as zidovudine, has a major impact on treatment regimens with a backbone of new NRTIs.

Furthermore, infections with revertants or atypical variants may also be relevant. Although in general they are not associated with reduced drug susceptibility, variants at position 215 of RT may lead to an increased ability to select resistance mutations as the genetic barrier to resistance is lowered.[33] The influence of 215 variants on virological outcome was retrospectively investigated in the Italian Cohort Naive Antiretrovirals (ICONA) cohort. An increase in virological failure was observed in seroconverters with a 215 variant at baseline after initiation of a thymidine analogue-containing regimen (47% versus 30% among those without a 215 variant).[34]

The presence of revertants and atypical variants may also be an indicator of transmission of a more extensive resistance profile, as illustrated by Van Laethem *et al*.[61] They reported a distinctive case in which baseline population gene sequencing identified one major resistance mutation and one atypical variant. Therapy was selected using a genotypic interpretation algorithm, but no response was seen. Retrospective analysis using more sensitive sequencing techniques revealed the presence of additional resistance mutations in minority variants. These mutations were rapidly selected, resulting in virological failure.[61] This case illustrates that resistance profiles based on population sequencing might be the tip of the iceberg (Figure 6). The possibility of more extensive underlying resistance should always be considered when selecting therapy in cases with transmitted resistance.

Comprehensive detection of transmitted drug resistance

In an effort to increase the detection of archived resistance mutations, parallel analysis of DNA from PBMCs has been performed by several groups. In a study of 169 treatment-naive HIV-1-infected individuals, Vicenti *et al*.[62] performed population sequence analysis on both plasma RNA and PBMC DNA. Although the overall results did not show increased sensitivity, in some cases additional drug resistance mutations were found in PBMCs. Two groups have looked into transmitted resistance in both compartments. Comparing evolution in the two compartments showed no differences in 3/5 cases, while in two patients 215Y and C mixtures were detected earlier in either plasma or PBMCs.[27] Another group only looked into PBMCs at the end of follow-up, revealing longer persistence of the RT mutation M184V in PBMCs.[24] Although these data are limited, they suggest that circulating PBMCs do not always serve as a representative archive for transmitted resistant HIV.

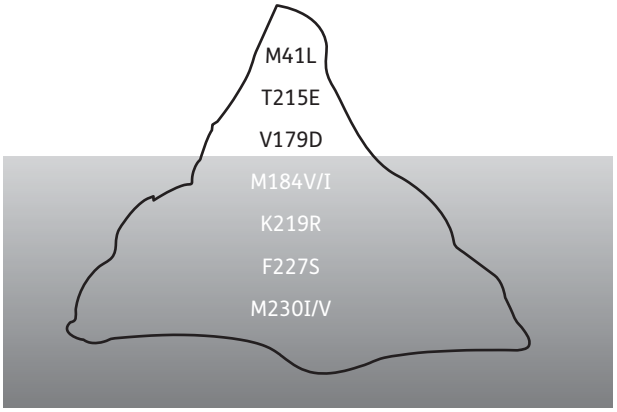

Figure 6. 'Iceberg' model reflecting drug resistance mutations in HIV-1 quasispecies. Only some of the mutations in the quasispecies are detectable above the water level, while minority variants harbouring additional mutations are below the surface and are not detected by population-based sequencing.

Additionally, clonal sequencing can also provide more insight into viral minorities present in the quasispecies. This type of analysis revealed gradual reversion towards wild-type for some drug resistance mutations.[24,28,31]

New, even more sensitive techniques have provided more insight into the presence of resistant minority variants in plasma. Johnson *et al*.[63] describe the use of a real-time PCR-based assay that is able to detect several minority drug resistance mutations (PR mutation L90M and RT mutations M41L, K70R, K103N, Y181C, M184V and T215F/Y). In a cross-sectional analysis the sensitive PCR identified one or more minority drug resistance mutation(s) in 34/205 (17%) of newly diagnosed individuals who were first considered to be infected with drug-susceptible HIV-1 based on conventional population sequencing. The clinical relevance of their observation was confirmed in a case–control study: 7/95 (7%) of those who experienced virological failure had minority drug resistance mutations at baseline compared with only 2/221 (0.9%) of treatment successes. It is noteworthy that all patients were started on an NNRTI-based regimen. These data suggest that a considerable proportion of transmitted HIV-1 drug resistance goes undetected by conventional genotyping and that isolated minority mutations can have clinical consequences if a regimen with a low genetic barrier is initiated.[63]

Discussion

Based on the available literature, we identified three different evolutionary pathways. Rapid evolution towards wild-type is observed for several drug-resistant variants with a profound

effect on RC. Incomplete evolution towards wild-type may be observed if intermediates between the drug-resistant variant and wild-type are generated that have an RC that almost equals wild-type. A second pathway is evolution to atypical variants. These atypical variants do not represent intermediates, but equivalently result in a higher RC than the original transmitted resistant variant. Finally, persistence of drug-resistant variants may be observed due to several underlying mechanisms. Mutations that induce only a limited decrease in RC often tend to persist. Furthermore, persistence of drug-resistant variants may also occur despite a reduced RC due to compensatory fixation. Such compensatory mutations may also be selected after transmission as additional mutations.

It is plausible that viral variants with a profound effect on RC are transmitted less frequently,[64] but no convincing evidence has been provided yet. This review shows that the relative underrepresentation in newly diagnosed patients of mutation M184V in RT, which is among the most common mutations in treated patients,[65] can be explained at least partially by rapid reversion during follow-up according to this first evolutionary pathway.[66]

It should be noted that there is considerable variability in the way the data were generated in the different studies. Several studies started the follow-up at the time of primary infection or directly after seroconversion while others determined the genotype later (2–16 weeks after seroconversion). The length of the follow-up after seroconversion varied from 2 to 58 months (median 18 months) and the interval between different genotypic analyses differs in each study. These variations make it difficult to make an estimate of the average time over which mutational patterns persist. In addition, several studies did not account for the presence or additional selection of secondary resistance mutations. They can significantly alter the RC of viruses harbouring primary resistance mutations and may therefore play a pivotal role in evolution after transmission. Despite these methodological variations and the limited availability of data, the general trends of evolution of mutations were very similar in all reviewed papers.

Selective pressure by the immune system may also influence the evolution or persistence of transmitted resistance profiles. Since immune pressure is primarily exerted on the envelope and Gag and there is only a limited number of epitopes related to drug resistance positions in PR and RT, the effect of the immune system on the viral evolution patterns covered in this review is most likely limited.[67]

There is currently only limited information available on the clinical implications of transmitted drug resistance. Applying genotypic analysis prior to first-line therapy may detect drug resistance and may prevent virological failure. However, traditional genotyping even in combination with PBMC analysis does not always reveal the full pattern of transmitted resistance. Evidence is mounting that more extensive resistance can be detected using more sensitive techniques and the presence of major mutations as minority species at baseline can diminish the treatment response, particularly to a regimen with a low genetic barrier.[63,68–70] Individualized resistance testing and therapy, with the preferential use of regimens with a high genetic barrier, may be of value in preventing failure of first-line therapy after infection with drug-resistant HIV-1.

In conclusion, different pathways of evolution of transmitted resistance patterns are observed. Data are being accumulated to show that specific transmitted drug-resistant viruses can persist and onward transmission of these variants to new hosts may occur. It is therefore important to continue monitoring the appearance of drug-resistant viruses in populations on treatment and the rate of transmission in newly diagnosed patients.

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Transparency declarations

None to declare.

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