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Worldwide Transmission of Drug-resistant HIV

Annemarie M.J. Wensing[1,2] and Charles A.B. Boucher[1]

[1]Eijkman Winkler Center, Department of Medical Microbiology, Division of Virology and [2]Department of Internal Medicine, Division of Acute Medicine and Infectious Diseases. University Medical Center, Utrecht, The Netherlands

Abstract

The availability of highly active antiretroviral therapy (HAART), that suppresses replication of the human immunodeficiency virus type 1 (HIV-1), has dramatically improved the prognosis of HIV-infected patients[1]. In populations with access to HAART, the course of the infection has changed from an inevitably fatal disease, characterized by a high incidence of opportunistic infections, into a potentially-treatable chronic condition. Unfortunately, HAART does not durably suppress HIV replication in 20-50% of treatment-naive patients and in up to 50-70% of treatment-experienced patients[2-4]. In the majority of patients with viral rebound, drug-resistance-related mutations are detected[5-9].

New infections through transmission of drug-resistant strains to individuals who have never been exposed to therapy are now being increasingly reported, despite all HIV prevention efforts. Moreover, recent reports correlate new infections by drug-resistant virus with suboptimal therapy response, which raises major public health concerns[10-13]. Despite a large number of publications on the rate of primary resistance, it is very difficult to draw general conclusions. The large variation in methodology and interpretation illustrates the need for systematic approaches. Global surveillance is urgently warranted to monitor the circulating HIV-strains. In addition, follow-up research has to be performed to reveal the impact of drug resistance on future therapy options. This paper reviews current literature to elucidate the mechanisms, implications and magnitude of transmission of drug-resistant HIV-1.

Key words

HIV. Drug resistance. Transmission. Epidemiology. Antiretroviral drugs.

Correspondence to:
Annemarie M.J. Wensing
Eijkman Winkler Center
Department of Medical Microbiology
Division of Virology
University Medical Center
Utrecht, The Netherlands
E-mail: A.M.J.Wensing@lab.azu.nl

Viral heterogeneity and development of drug resistance

Infection with HIV is characterized by the extreme genetic diversity of the virus population. Continuous high replication[14] and a high error rate of HIV-1 reverse transcriptase result in a swarm of genetic viral variants, called quasispecies. Additionally, recombination between viral variants may contribute to the heterogeneity of the quasispecies. Due to the genetic diversity of the HIV-infection, variants with every possible point mutation are present in the quasispecies[15]. The variants continuously evolve and are capable of evading the host immune responses, acquiring altered cell tropism and presenting drug-resistant variants. The heterogeneity of the HIV-1 population within an infected individual may not be evenly distributed throughout the body. Disparity has been found between viral quasispecies and the quantity of HIV-RNA in different parts the body, particularly in the brain, blood, genital tract and lymphoid tissue[16-19].

Under pressure of therapy, pre-existing variants with a reduced susceptibility can be selected and achieve dominance. Use of NRTI-mono- and duotherapy often results only in suboptimal suppression of the HIV-replication, allowing rapid evolvement of resistance[20]. Combining at least three drugs in HAART increases the genetic barrier to resistance, and is capable of achieving suppression of circulating virus below the limits of assay detection. As a result, HIV-related morbidity and mortality have declined impressively[1].

However, new major obstacles have arisen. Complex intake regulations, high pill-burden and substantial toxicity complicate strict therapy adherence. Variations in pharmaco-dynamics between individuals, and differential penetration of drugs in sanctuary sites, may lead to sub-therapeutic plasma levels. Furthermore, pre-existing drug-resistance-related mutations, either resulting from natural polymorphisms in the quasispecies or from transmission by treated patients, might compromise drug susceptibility even before initiation of therapy. As a result of these obstacles, HAART does not durably suppress HIV-replication in 20-50% of treatment-naive patients and in 50-70% of treatment-experienced patients[2-4]. Detectable viral replication during antiretroviral therapy favors the selection of viruses that carry or develop resistance-related mutations. Hence, it is not surprising that, in a majority of patients with therapy failure, drug-resistant variants are detected[5-9].

Definitions of drug resistance

The terminology that is used in the field of transmission of resistance may be confusing. Resistance is frequently divided into major (or primary) resistance mutations and secondary (accessory or compensatory) resistance mutations. Major mutations are resistance-related mutations that are selected under drug pressure. They have often a profound effect on viral drug susceptibility. The presence of a major mutation results, in most cases, in an impaired fitness of the viral strain compared to wild-type in an environment without drug pressure. As a result, major drug-resistant mutations may exist in viral quasispecies that have never encountered therapy, but not at a frequency enabling detection by resistance assays[15,21]. Alternatively, viral quasispecies that have encountered therapy in one host, and that are subsequently transmitted to a new therapy-naive host, can harbor major drug-resistant mutants in a drug-free environment.

Secondary resistance mutations are defined as having little or no effect on drug susceptibility, but are selected under drug pressure to compensate for the changes caused by primary mutations. Variants with secondary mutations may also be detected as natural polymorphisms in the quasispecies, particularly in the protease gene.

Primary resistance, or baseline resistance, accounts for drug resistance that is present before initiation of therapy. Primary resistance may be present as a natural variation due to the heterogeneity of the virus or may be the result of transmitted resistance that was selected during therapy in another patient. In antiretroviral-naive patients, it may be very difficult, and sometimes impossible, to differentiate whether secondary mutations result from a natural variation in the quasispecies or from transmission after selection under therapy in another host. Therefore, most reports describing transmitted resistance only take into account major mutations, since their presence in untreated subjects clearly indicates transmission of resistance (Tables 1-6).

Evolution of drug resistance in treatment-naive patients

In treated patients carrying drug-resistant HIV variants, cessation of the complete regimen usually results in a rapid reappearance of the original drug-sensitive wild-type[22]. However, resistant variants may persist as minority populations in plasma and are maintained as provirus in resting cells within lymphoid tissue. As soon as therapy is reintroduced, the resistant variants can be rapidly reselected[23].

In antiretroviral-naive individuals, wild-type may dominate resistant variants with impaired fitness, if the infecting quasispecies contained both wild-type and resistant variants. If, however, through a founder effect, the diversity of infecting quasispecies is restricted to resistant variants, and no minority of wild-type variants is present, dominance by wild-type is not possible. In the absence of drug pressure, the transmitted drug-resistant quasispecies may revert back to wild-type or mutate to other more fit variants. Viruses harbor-

Table 1.

Primary drug resistance reported in Europe								Genotypic resistance						Phenotypic resistance					
Author	**Country**	**Area**	**Duration infection**	**Risk-factor**	**Sub-type**	**Year**	**N**	**NRTI**	**NNRTI**	**Major PI**	**Sec PI**	**Tot Prim**	**>1 class**	**fold**	**NRTI**	**NNRTI**	**PI**	**Tot**	**>1 class**
Van Vaerenbergh, et al.[118]	Belgium	Multiple areas		MSM HSX		'98	231	11%#	16%#	10%#		32%#	3%#						
						'97		15%#	13%#	8%#		32%#	4%#						
						'95		16%#	18%#	4%#		27%#	4%#						
Descamps[113]	France	Multiple areas	Chronically infected	MSM HSX IDU	90% B	'98	391	3%	1%	2%	49%	4%							
Harzic[11]	France	Multiple areas	<3 months	MSM HSX		'99	205	10%		8%									
						'98			3%	3%									
						'97			7%	4%									
						'96			11%	0%									
Duwe, et al.[137]	Germany	Berlin/Hamburg	<3 years	MSM	B	'96-'99	64		9%	5%	100%								
Magiorkinis, et al. [112]	Greece				76% B	'99-'00	25	0%	0%	0%	91%	0%							
Perno, et al.[114]	Italy	ICONA cohort	>1 year	IDU HSX MSM		Before '02	347	8%	5%	1%	78%		10%						
Romano, et al. [116]	Italy	Tuscany	<2 years	MSM HSX	93% B	'96-'00	116	13%	1%	1%	78%								
De Mendoza, et al.[138]	Spain	Multiple areas	<12 months	MSM HSX		'00-'01	57	0%	0%	4%		4%							
						'97-'99				26%		26%							
Gallego, et al.[133]	Spain	Multiple areas	>2 years			'00	130	3%	2%	6%	11%	0%							
Puig, et al.[117]	Spain	Multiple areas				'98	59	17%#		6%#			2%#						
Horban, et al.[111]	Poland	Warsaw	Therapy naive	IDU SEX		'00-'01	128	52%#											
Yerly, et al.[48]	Switzerland	Swiss cohort	<3 months	MSM HSX IDU		'96-'98	82	10%	2%	4%									
Fidler[119]	UK	London	<6 months	SEX	87% B	'00-'01	28	0%	0%	0%		0%							
UK- register[120]	UK	Multiple areas	<18 months		87% B	'00	69	17%	12%	4%		27%							
						'94-'99		7%	0%	0%		7%							
Geretti[76]	UK		Established infection	HSX	38% C 34% B	'99-'01	72	2%	3%	4%	100%	6%	3%						

NRTI = nucleoside reverse transcriptase inhibitor; NNRTI = non-nucleoside reverse transcriptase inhibitor; PI = protease inhibitor; Major = major resistance related mutations; SEC = secondary resistance related mutations; fold = fold resistance; Tot = total; # Use of a hybridization assay that detects a specific subset of mutations; CC = clinical cut-off

Table 2.

Primary drug resistance reported in North America								**Genotypic resistance**						**Phenotypic resistance**					
Author	**Country**	**Area**	**Duration infection**	**Risk-factor**	**Sub-type**	**Year**	**N**	**NRTI**	**NNRTI**	**Major PI**	**Sec PI**	**Tot Prim**	**>1 class**	**fold**	**NRTI**	**NNRTI**	**PI**	**Tot**	**>1 class**
Health Canada[123]	Canada	3 regions	Newly diagnosed	MSM HSX IDU		'97-'00	563	4%	0%	2%		6%	0%						
Salomon, et al.[91]	Canada	Vancouver	PHI <3 months	MSM IDU		'97-'99	81	19%		6%	~75%	–	10%	Only subset					
Alexander, et al.[122]	Canada	British Columbia	Therapy naive			'97-'98	479	3%		4%	>50%	6%							
Alexander, et al.[90]	Canada	British Columbia	PHI <6 months	IDU		'96-'98	61	0%		2%		2%							
Escoto-Delgadillo, et al.[139]	Mexico	8 Mexican centers	Therapy naive			'01	60	4-5%	5-7%	2%									
Grant, et al.[12]	USA	San Francisco	<1 year			'00-'01	248	21%	13%	8%		27%	13%	>10 CC	5%-6%	10% =	6%-6%		16%
						'98-'99		7%	6%	5%		18%	1%	>10 CC	0%-3%	4% =	0%-2%		10%
						'96-'97		25%	0%	3%		25%	3%	>10 CC	8%-21%	0% =	3%-3%		21%
Ristig, et al.[140]	USA	St. Louis	>6 months <5 years	MSM HSX		'99-'01	35	9%	6%	6%		17%							
						'96-'98	27	4%	0%	0%		4%							
Little, et al.[10]	USA	North America	<1 year	MSM		'99-'01	377	16%	7%	9%		23%	10%	>10	6%	7%	8%	12%	6%
						'95-'98		9%	2%	1%		8%	4%	>10	2%	2%	0%	3%	1%
Simon, et al.[121]	USA	New York	<3 months	MSM	B	'99-'01	154	15%	7%	5%	80%	20%	4%	CC/2.5	3%	8%	5%	11%	4%
						'95-'98		12%	3%	1%	76%	13%	3%	CC/2.5	8%	5%	2%	10%	3%
Sullivan, et al.[141]	USA	Seattle LA	<2 year or CD4 > 700	MSM	B	'97-'99	95	1%	3%	0%	94%	4%		Only subset					
Wegner, et al.[142]	USA	US military	<3 years			'97-'98	114	1%	6%	1%	8%			5-10 >10	7%-1%	19%-8%	0%-1%		3%

NRTI = nucleoside reverse transcriptase inhibitor; NNRTI = non-nucleoside reverse transcriptase inhibitor; PI = protease inhibitor; Major = major resistance related mutations; SEC = secondary resistance related mutations; fold = fold resistance; Tot = total; CC = clinical cut-off

Table 3.

Primary drug resistance reported in South America								Genotypic resistance						Phenotypic resistance					
Author	**Country**	**Area**	**Duration infection**	**Risk-factor**	**Sub-type**	**Year**	**N**	**NRTI**	**NNRTI**	**Major PI**	**Sec PI**	**Tot Prim**	**>1 class**	**fold**	**NRTI**	**NNRTI**	**PI**	**Tot**	**>1 class**
Kijak, et al.[124]	Argentina	Buenos Aires	Therapy naive	SEX IDU		'97-'00	94	1%	0%	1%									
			PHI	SEX		'99	13	8%	0%	8%									
Tanuri[125]	Brazil	Multiple areas			55% B 26% C 9% F	'02?	154	2%	4%	1%									
Varella, et al.[126]	Brazil	Rio de Janeiro	Recent 10 Chronic 29		87% B	'02?	39	5%	5%	0%		10%							
Leal, et al.[143]	Brazil	Sao Paulo	Therapy naive		88% B 12% F	'00?						11%							
Sabino, et al.[144]	Brazil	Sao Paulo	Therapy naive			'95-'96	59	14%#											

NRTI = nucleoside reverse transcriptase inhibitor; NNRTI = non-nucleoside reverse transcriptase inhibitor; PI = protease inhibitor; Major = major resistance related mutations; SEC = secondary resistance related mutations; fold = fold resistance; Tot = total; CC = clinical cut-off

Use of hybridization assay that detects a specific subset of mutations.

Table 4.

Primary drug resistance reported in Australia								Genotypic resistance						Phenotypic resistance					
Author	**Country**	**Area**	**Duration infection**	**Risk-factor**	**Sub-type**	**Year**	**N**	**NRTI**	**NNRTI**	**Major PI**	**Sec PI**	**Tot Prim**	**>1 class**	**fold**	**NRTI**	**NNRTI**	**PI**	**Tot**	**>1 class**
Ammaranond, et al.[131]	Australia	Sydney	PHI	MSM		'96-'01	185	9%		0%									
						'92-'95	185	29%		2%									

NRTI = nucleoside reverse transcriptase inhibitor; NNRTI = non-nucleoside reverse transcriptase inhibitor; PI = protease inhibitor; Major = major resistance related mutations; SEC = secondary resistance related mutations; fold = fold resistance; Tot = total; CC = clinical cut-off

Table 5.

Primary drug resistance reported in Asia								Genotypic resistance						Phenotypic resistance					
Author	**Country**	**Area**	**Duration infection**	**Risk-factor**	**Sub-type**	**Year**	**N**	**NRTI**	**NNRTI**	**Major PI**	**Sec PI**	**Tot Prim**	**>1 class**	**fold**	**NRTI**	**NNRTI**	**PI**	**Tot**	**>1 class**
Cho, et al.[127]	Korea	Ulsan	Chronic		79% B	'02?	40			2%									

NRTI = nucleoside reverse transcriptase inhibitor; NNRTI = non-nucleoside reverse transcriptase inhibitor; PI = protease inhibitor; Major = major resistance related mutations; SEC = secondary resistance related mutations; fold = fold resistance; Tot = total; CC = clinical cut-off

Table 6.

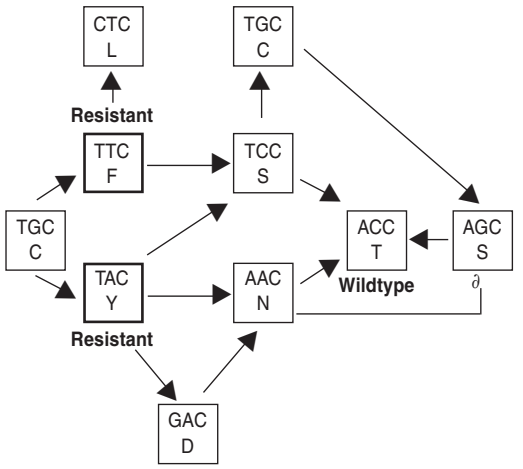
Primary drug resistance reported in Africa								Genotypic resistance						Phenotypic resistance					
Author	**Country**	**Area**	**Duration infection**	**Risk-factor**	**Sub-type**	**Year**	**N**	**NRTI**	**NNRTI**	**Major PI**	**Sec PI**	**Tot Prim**	**>1 class**	**fold**	**NRTI**	**NNRTI**	**PI**	**Tot**	**>1 class**
Bussmann, et al.[72]	Botswana	National sites	Therapy naive		C	'01	51		2%	4%	80%								
Toni, et al.[128]	Ivory Cost	Abidjan	<3 years		83% CRF02 AG	'97-'00	99	0%	0%	0%	>90%	0%							
Petch, et al.[129]	Malawi		Therapy naive		C	'02?	21	0%	0%	0%	>90%	0%							
Tarin, et al.[130]	South-Africa	KwaZulu Natal	Therapy naive		C	'02?	46	0%	4%	0%	>90%	7%							
Handema, et al.[73]	Zambia		Therapy naive		93% C	'00	28	0%	0%	0%	>90%	0%							

NRTI = nucleoside reverse transcriptase inhibitor; NNRTI = non-nucleoside reverse transcriptase inhibitor; PI = protease inhibitor; Major = major resistance related mutations; SEC = secondary resistance related mutations; fold = fold resistance; Tot = total; CC = clinical cut-off

ing a single resistance-related point mutation, such as M184V in the reverse transcriptase (RT) gene, may revert more rapidly than variants that have evolved after multiple mutations[24]. Also, persistence of transmitted resistant variants for several months or years in the plasma of the newly infected subject has been described[25,26].

Furthermore, intermediate stages between resistant variants and wild-type or atypical variants are occasionally detected at codon 215 of viruses isolated from antiretroviral-naive patients[27-29]. These variants emerge from the resistance-related mutants T215Y and T215F, which need two nucleotide changes for reversion to wild-type[13,27,28] (Fig. 1). Viruses containing these partial revertants or atypical mutations are selected because they display an increased fitness compared to the resistant variants. *In vitro* experiments have shown that variants with the atypical mutation 215D, or partial revertant 215S, can replicate as efficiently as wild-type, which may explain their persistence *in vivo*[27,28]. Although the partial revertants or atypical mutants do not, by themselves, confer phenotypic resistance, they are only one step away from the resistant variants 215Y or 215F, compared to the two mutational steps that are needed from wild-type to 215Y/F. This may indicate an increased risk for developing resistance under subsequent treatment with zidovudine or stavudine[28].

In the absence of selective drug pressure in the new host, resistant variants may completely revert to wild-type, leaving no evidence of transmitted resistance in the plasma. However, one might expect that the initial resistant quasispecies will still be present as archived provirus in resting cells of the untreated subject.



Resistant codons are F = phenylalanine and Y = tyrosine. Wild-type is T = threonine. Intermediate stage are S (TCC) = serine and N = asparagine. Atypical variants are D = aspartic acid, S (AGC) = serine and L = leucine[27-29]

Figure 1. *Possible evolutionary pathways of mutant codon 215.*

Detection of drug resistance in treatment-naive patients

Several assays are used to assess antiretroviral drug resistance. Phenotypic drug-susceptibility assays determine the concentration of an antiretroviral agent that is required to inhibit HIV-1 replication by 50% (IC_{50}) or 90% (IC_{90}). The results are compared to the concentration of drug necessary to inhibit a reference strain of susceptible virus. The difference in IC_{50} or IC_{90} defines the susceptibility of the virus to the different antiretroviral agents. Genotypic resistance tests identify nucleotide changes in the viral genome compared to a viral reference strain. The profile of mutations is interpreted using algorithms that correlate known mutational profiles to phenotypic susceptibility data and/or clinical outcome. Both tests have shown their benefits in the clinical practice of treatment-experienced HIV-infected patients[30,31], and may become of clinical value for antiretroviral-naive individuals too. However, several specific issues need attention when susceptibility testing is applied to detect transmission of drug-resistant viruses.

Phenotypic resistance tests and genotypic sequence analyses are generally capable of identifying viral populations that make up at least 20% of the quasispecies. Transmitted resistant variants, partly overgrown by or reverted to wild-type may, therefore, be missed, suggesting that these tests might underestimate the presence of transmitted drug-resistant viruses. More sensitive resistance tests, such as the Line Probe assay, may be very useful for this indication, but have the drawback of only considering a limited amount of resistance-related positions and frequent hybridization failure[32,33].

While phenotypic susceptibility assays can be used to measure the actual susceptibility of viral strains, genotypic sequence analyses are sometimes able to trace the evolutionary pathways of transmitted drug resistance. Genotypic tests may, therefore, identify mutations or genetic profiles in treatment-naive patients that are not necessarily resulting in phenotypic resistance but may furnish evidence for transmission of resistant variants in the past. This is of particular interest for epidemiological research, although it might become clinically relevant too, since there are indications that certain transmitted mutational profiles may facilitate the development of drug resistance under selective pressure[27,28].

Mechanism of HIV-1 transmission

Individuals may be exposed to varying amounts of virus depending on the route of HIV-1 transmission. Sexual and vertical transmission generally involves smaller amounts of virus and infected cells than transmission due to direct blood-to-blood contact as occurs during intravenous drug use or blood transfusion[34-36].

Regardless the route, transmission is accompanied by a loss of both genotypic and phenotypic viral heterogeneity[37]. As a result, the viral population proliferating in the blood of recently-infected recipients is generally more homogeneous than the markedly heterogeneous quasispecies in chronically-infected donors[34,37-39]. The restriction in quasispecies implies the occurrence of a bottleneck process during transmission and establishment of infection. This process is determined by the number of transmitted virions, by the diversity of the transmitted viral quasispecies and by host factors, such as the mucosal barrier, the density of target cells and the immune system[40].

Recent studies suggest that multiple strains are frequently transmitted, resulting in more genomic variation in the recipient than previously reported, though still not as heterogeneous as the donor population[35,41]. Subsequently, variants among the transmitted population that are most capable of widespread dissemination might be positively selected[41]. The viruses that succeed in establishing infection do not have to be the variants that displayed the highest fitness under drug pressure in the donor quasispecies –as demonstrated by the ability of minor variants from the donor quasispecies to accomplish infection in the recipient[34]. This finding might be explained by stochastic processes that influence the diversity of the viral variants in the inoculum. Another reason for a shift in quasispecies might be that characteristics preferred during chronic infection differ from viral properties selected during transmission and adaptation to the new host.

Transmission of drug-resistant variants

The first case of transmission of drug-resistant HIV was reported 10 years ago by Erice, et al.[42]. A patient who had never been exposed to therapy carried a viral variant resistant against zidovudine, not reflecting a natural variation in drug susceptibility. A few years after the introduction of combination therapy, Hecht, et al. described the transmission of an HIV variant resistant to multiple RT and Protease inhibitors[43]. Since then, transmission of virus resistant to antiretroviral drugs has been repeatedly documented through many infection routes: sexual contact, needle sharing among intravenous drug users, perinatal transmission and accidental exposure of health care workers[44-47]. More recently, cohort studies have been published describing patterns of baseline drug-resistance profiles in a significant percentage of therapy-naive patients[48-50].

Multiple studies have reported impaired enzyme function and decreased viral fitness of HIV-1 isolates harboring drug-resistant mutations, often resulting in a moderate or low viral load in the patient[51-53]. One might speculate that these strains are also less capable of accomplishing infection in a new host. The high frequency of primary resistance in some recent reports seems, however, to argue against reduced infectiousness of these viral strains[54]. On the other hand, if the transmitted quasispecies also contains drug-sensitive variants, resistant variants with impaired fitness may be at selective disadvantage during infection and/or initial amplification in the new host[55,56].

Quite interesting in this perspective, is a recently published mathematical transmission model studying primary resistance in recent seroconverters. It suggests that the frequency of primary resistance in seroconverters is lower than expected, based on the prevalence of resistance in the population of potential transmitters[57]. In addition, another report states that primary resistance to more than one class of drugs is not as common as may be expected, based on the rate of transmission of resistance and the frequency of drug resistance in the population[58]. It is not entirely ruled out that the differences in frequency of resistance between the populations might be related to a lower engagement of risk behavior of treatment-experienced patients, or to a loss of transmitted drug resistance in the new host in the short period after infection. However, the authors conclude that drug-resistant viruses have a lower transmission fitness than drug-sensitive viruses[57]. Another suggested possibility is that individuals carrying drug-resistant variants frequently transmit drug-sensitive variants[57]. A third explanation is that it is too early in the epidemic to observe a high rate of transmission of (multi-)drug-resistant viruses. It might be that predominantly drug-sensitive or NRTI-resistant viruses from the early treatment period are circulating in current transmitters.

Transmitting population

Transmission of HIV-1 is strongly influenced by the level of viremia in the HIV-infected donor[59-61]. Consequently, it seems logical to relate the rate of transmission of HIV to the overall level of viremia in the HIV-infected population. In this perspective, access to HAART has, by reduction of HIV-RNA concentrations, the potential to decrease transmission of HIV on a population level[59,62]. In pregnant HIV-infected women, the use of HAART has indeed shown a profound reduction of HIV incidence in their off-spring. Despite this success in preventing vertical transmission, HAART is currently not effective as a “population prevention measure”. In contrast, prolonged survival of infected patients, continuous injecting drug use and sexually related transmission, have resulted in an increased prevalence of HIV-infections in countries with access to HAART[63,64]. Although many patients have adopted safer practices after they became aware of their infection, some are still engaged in risk-related behavior as reflected by the transmission of drug-resistant HIV to individuals who have not yet been exposed to therapy.

The impact of HAART on the spread of HIV and its drug-resistant variants may vary between dif-

ferent transmitters. For instance, the beneficial effect of therapy on HIV-RNA load will not reach individuals who are unaware of their infection status. Persons with primary infection among this population may, however, be very infectious, because viremia in the plasma is extremely high during the acute phase of infection[65]. In contrast, the viremia in semen during acute infection appears not to be different from that during established infection, although this has only been studied to a limited extent[66]. Nevertheless, data from the Swiss cohort prescribe an important role of primary infection on the spread of HIV-1. One-third of acutely-infected patients in the cohort appeared to be infected by persons who had acquired HIV very recently themselves[67]. In addition, they identified five cases of sexual transmission during the incubation period of primary infection[68]. Although seroconverters are rarely assumed to be the source of drug-resistant viruses, secondary transmission of resistant virus has been described in recent HIV infection and may become more frequent in the future[64].

Other possible transmitters are chronically-infected individuals that have been diagnosed with HIV, but not yet exposed to therapy. Toxicity and complexity of the current antiretroviral regimens have resulted in new guidelines favoring less aggressive strategies for initiating therapy in this population. This more conservative approach is leading to an increased number of individuals enduring unsuppressed viremia. Since a significant dose-response relation between transmission and the level of viral load has been described, this new strategy may lead to an increased risk of transmission at the population level[59]. If these antiretroviral-naive persons have been infected many years ago before the widespread use of antiretroviral therapy, they are, however, unlikely to play a significant part in the spread of drug-resistant viruses, since drug-resistant viral variants were probably not circulating at a high prevalence by then.

Antiretroviral-experienced individuals make up a third group of possible transmitters. Ideally, persons taking antiretroviral therapy should be less efficient in transmitting HIV, at least if their viral load is successfully suppressed[59]. Unfortunately, in many individuals on HAART, the viral load is not below the detection of the commercially available assays[2,4]. Furthermore, the limited amount of therapeutic options supports continued use of HAART regimens that, even though unsuccessful from a virology point of view, are capable of sustaining a prolonged immunologic response. Since patients experiencing viral rebound frequently harbor drug-resistant variants, they are the most likely source of transmission of resistant viruses. However, it has been suggested that the frequency of transmission of drug-resistant viruses is less than expected, due to their impaired viral fitness.

Different models have been proposed to predict which populations will drive the spread of drug-resistant strains in the future[69,70]. The models are based on data from different cohorts and have a completely different outcome. The first model that was based on a small cohort, with low frequency of transmission of drug-resistance, indicates that the frequency of viral resistance in a population is determined largely by the number of individuals on insufficient or failing therapy, and will be influenced only modestly by secondary transmission of drug-resistant strains[69]. The outcome of the second model, which was based on a population with a higher frequency of primary resistance, implies that secondary transmission by antiretroviral-naive patients will become the major source of new infections with drug-resistant variants[70].

Transmitted resistance in non-B subtypes

HIV-1 can be divided into three distinct and highly divergent groups (M, N and O). Globally, HIV-1 M is the most prevalent and is classified into a variety of subtypes[71]. To date, almost all information on HIV-1 drug resistance is based on studies performed on subtype B, which was originally the most predominant subtype in Western Europe, Australia and the United States. However, the majority of HIV-1 infections worldwide are caused by non-B subtypes, particularly subtype A and C. Reports on the characterization of non-B sequences derived from untreated subjects describe a very low frequency of transmitted primary resistance-related mutations, reflecting the limited access to therapy in areas where these subtypes circulate[72,73] (Tables 1-6). In contrast, secondary resistance-related mutations are observed at a very high frequency as naturally occurring polymorphisms in non-B subtypes[73-75]. As a consequence, it may be extremely difficult to distinguish in non-B subtypes between natural polymorphisms and (transmitted) secondary mutations resulting from selective drug pressure[76]. Hence, collection and characterization of circulating non-B sequences in areas before introduction of therapy is tremendously important to enable the future evaluation of treatment programs and surveillance of transmitted drug resistance.

Initiatives to implement therapy in resource-limited countries have been criticized because of fear of massive development of drug resistance in settings with sub-optimal health care facilities. Surveillance programs need to be installed to gain a reliable insight into the future spread of drug-resistant strains, and to create the possibility for early adjustment of treatment guidelines if necessary.

In addition, there is increasing evidence that, in some cases, HIV subtypes may have distinct pathways to resistance. For instance, the V106M mutation selected by efavirenz is associated with a higher prevalence in non-B subtypes and may arise from a more efficient pathway then that observed in B subtypes[77]. Comparable reports have been published about nelfinavir-related re-

sistance[78]. Algorithms for the interpretation of (transmitted) resistance profiles should take these specific evolution pathways into consideration.

Sexual transmission

Worldwide, heterosexual contact is the predominant mode of HIV-1 transmission. Although it seems more logically to relate sexual transmission to the concentration of HIV in the genital tract, for reasons of convenience the plasma viral load has been most frequently identified as the major predictor of the risk of sexual transmission of HIV-1[59]. Despite the frequent correlation of HIV-RNA levels between the two compartments, differences are observed[79], especially in case of local inflammation and ulceration, which may significantly increase the amount of HIV-RNA in genital fluids and also the susceptibility to infection[80]. Generally, however, initiation of HAART leads to a reduction of HIV-RNA in genital secretions of both men and women parallel with the decline seen in the plasma[81,82]. In persons with a plasma viral load of <1500 HIV-RNA copies/ml, sexual transmission is uncommon[59]. Nevertheless, absence of detectable HIV-RNA in plasma and genital secretions does not prove that individuals cannot transmit HIV. Even if HIV-RNA is completely absent in genital fluids, individuals may possibly transmit cells that contain proviral DNA[81,83].

Also, the rate and pattern of the emergence of resistance in the genital fluids can be different from that in the systemic circulation[83-85]. These compartments are, therefore, frequently considered as biologically separate sites[82,84-86], in which viral replication and evolution are exposed to different selective pressures than in the systemic circulation[39]. One determinant of differential virus evolution may be the poor penetration of some antiretroviral drugs in the genital tract[87]. Low regional drug levels of protease inhibitors may, for example, lead to double-nucleoside therapy in these compartments, favoring development of resistance.

Because of the major consequences for transmission of (drug-resistant) HIV, the possibility of differential expression of HIV-1 quasispecies and viral load in the genital tract should always be considered.

Transmission by intravenous drug use

Injecting drug use is an important mode of HIV transmission in many parts of the world. Injecting drug users (IDU) may be less likely to seek care, and less likely to receive antiretroviral agents even if they are in care[88]. In addition, dependence on addictive stimulants may not always easily be combined with the requirements for successful therapy, such as the need to take antiretrovirals according to a specific time schedule, often accompanied by strict dietary regulations[89]. Insufficient adherence may put IDUs on therapy at high risk for acquiring HIV drug resistance. Therefore, one might expect a higher rate of transmission of drug resistance in the IDU population. However, during an outbreak of HIV infection in 1996-1998 among IDUs in British Columbia, Canada, the frequency of transmitted drug resistance was very low (2%)[90]. A possible explanation may be a limited use of antiretroviral therapy among the IDU population, despite the free supply of antiretroviral agents in Canada.

Another possibility may be the nature of the outbreak, with seroconverters infecting seroconverters with drug-sensitive virus. A completely different picture was observed in a study performed in 1997-1999 in Vancouver among 81 recently-infected antiretroviral-naive patients. Twenty-three percent of IDUs (5 out of 21) carried major drug-resistance mutations compared to 9% of individuals infected by sexual contact (5 out of 56)[91]. In the Swiss Cohort, the prevalence of transmission of drug-resistant viruses was, in 1996-1999, higher in recently-infected IDUs (13%) and the homosexual group (11%) compared to the heterosexual group (6%). However, this difference may be partially explained by early initiation of treatment in the first two groups, and a high percentage of infections with non-B subtypes among the last group[67].

Mother-to-child transmission

Mother-to-child HIV-1 transmission may occur during the intrauterine or intrapartum periods, or postnatally through breast milk. There is evidence that the majority of HIV-1 transmission occurs at or around the time of birth[92,93], but it is unknown whether transmission at this time is predominantly established through a parenteral route (due to maternal-infant transfusion via the placenta during labor) or through a mucosal route (due to conjunctival, oral, or nasopharyngeal exposure to maternal cervico-vaginal secretions)[93]. On the whole, the maternal plasma viral load is a good predictor of vertical HIV transmission, but there is neither a level above which transmission always occurs nor a level below which transmission is never seen[94]. Furthermore, despite a general correlation between HIV-RNA in plasma and in the cervico-vaginal secretions, discordance has been reported. Since exposure to HIV-1 in the maternal genital tract during delivery appears to be an independent risk factor for intrapartum transmission, the plasma viral load may not be an effective indicator of risk in these discordant situations[95,96].

In the industrialized world, a dramatic decline in the rate of vertical HIV acquisition has been observed since 1994, when the results from the PACTG 076-study became available. This study demonstrated that the use of zidovudine (by the mother during part of the pregnancy and at labor and by her child for six weeks) could decrease perinatal transmission of HIV by 67%[97]. High level resistance to ZDV is unlikely to appear after such

a short period of ZDV therapy and is characterized by sequential development of mutations, leading to increasing levels of resistance[20,98]. In fact, neither in the PACTG 076 nor in another trial, high level resistance to ZDV was reported in both women and children[99,100]. Whether the presence of ZDV resistance in pretreated women leads to an increased frequency of perinatal transmission is not clear because of the low numbers of infected children[101].

Recently, cohort studies demonstrated that combination therapy with protease inhibitors has led to a further decrease of perinatal transmission to less than 2%[102,103]. In general, independent of their viral load at the time of delivery, women on antiretroviral therapy display very low rates of vertical transmission. This may reflect transplacental passage of the drugs, providing post-exposure prophylaxis to the fetus.

In the developing world, the HIV-NET 012 study demonstrated that a single oral dose of nevirapine (NVP) given to the mother at onset of labor, together with a single oral dose given to her newborn within 48-72 h of life, significantly reduced vertical HIV transmission in women who were receiving no other antiretroviral treatment[104]. The efficacy, simplicity and low cost of this regimen make it attractive for use in resource-limited settings. There is, however, substantial discussion about the impact of this approach on resistance. A single point mutation is enough to confer high-level resistance to NVP[105]. Virus with mutations that confer resistance to NVP pre-exist commonly in the maternal viral population, but may be not present in high enough frequency to be detected using current genotypic resistance assays[106]. In the HIV-NET 012 study it became apparent that a single dose of NVP may select for virus containing these mutations, and in 19% of women the frequency of mutant viruses was high enough to allow detection at six to eight weeks postpartum[107]. NVP resistance was detected even more frequently in infants, but different patterns were seen compared to the mothers. Since both mother and child received therapy, this is probably not indicative of vertical transmission of drug-resistant HIV[107]. The resistance mutations identified during the early postpartum period disappeared from detection in plasma within 12-24 months in the women and within weeks in the children. However, it is unlikely that viruses containing the resistance mutations are totally eliminated from the quasispecies.

The long-term clinical consequences of these drug-resistant mutations on subsequent prophylaxis or treatment are currently unknown. We might expect that the presence of drug-resistant virus in the mother increases the probability of vertical transmission, but this has not yet been convincingly demonstrated[108]. Although the evidence that resistant strains can be transmitted in the perinatal period is ubiquitous[109,110], the infectiousness of drug-resistant strains may be decreased compared to drug-sensitive variants. This is illustrated by case reports that describe selection against ZDV-resistance mutations during vertical transmission[56,101].

Characterization and time trends of reported transmitted drug resistance

Large variations are seen in reports from many European countries describing frequency of drug resistance in viruses isolated from both recently- and chronically-infected patients[11,111-116]. However, in most reports with high prevalence rates a different technique was used[111,117,118]. An exception is one report from the UK that shows considerable transmitted resistance identified by regular genotypic sequence analysis, but this is, however, not confirmed by another UK-study describing an identical population[119,120].

A high prevalence (20-27%) of major resistance-related mutations in patients infected for less than one year is described in recent reports from the United States[10,12,121]. These reports come mainly from big cities with large homosexual populations that have a long history of easy access to therapy. In contrast, most reports from Canada show a low prevalence of primary resistance, except for one report from Vancouver, which may represent a similar population as the reports from the US[91,122,123]. In South America, where therapy has been introduced more recently, the frequency of major resistance-related mutations varied between 1-2% to 14% detected in predominantly unspecified therapy-naive populations[124-126].

Only a limited amount of information is available from countries in Africa and Asia with large epidemics. The restricted access to therapy in these areas is reflected in the very low to zero percentage of major resistance-related mutations in antiretroviral-naive subjects[72,73,127-130]. The African reports, however, describe, a very high percentage of secondary mutations reflecting drug-resistance-related polymorphisms in subtype C.

In countries with a long tradition of access to therapy, NRTI-related mutations, especially zidovudine mutations, are more frequently identified than NNRTI- and protease-related mutations. This might reflect the extensive use of zidovudine monotherapy in the past. In addition, it might also point out the characteristic accumulation of resistance-related mutations that are selected by zidovudine. Reversion of this resistance profile in a drug-free environment might be more difficult compared to mutational profiles selected by other drugs, thereby allowing prolonged detection of zidovudine-related mutations.

The one report from Australia demonstrates a huge decrease in NRTI-related resistance from 29% before 1996 to 9% in more recent years[131]. Similar drops in prevalence of NRTI-resistance after the introduction of HAART have been reported by other groups[118,131-133]. The higher frequency of transmitted resistance against NRTIs in the pre-HAART area probably reflects less-effective viral suppression by mono- and duo-NRTI-therapy. NNRTI- and PI-related resistances have increased gradually since their introduction in these countries, but in general do not reach a rate above 10%.

Overall, individuals identified more recently after infection have the highest risk of carrying drug-resistant mutations. This may indicate either a current rise in transmitted resistance or a decrease in detectability of transmitted resistance in a drug-free environment over time. Finally, primary resistance to more than one class of drugs varied extensively between 0 and 16%, making it very difficult to draw specific conclusions on the frequency of transmission of multi-drug resistance.

Difficulties in assessing the rate of transmission of drug-resistant virus

In the currently available literature, different rates of transmission of resistant HIV are reported (Tables 1-6). These different rates may reflect variations in access to therapy, therapeutic strategies, adherence or risk-related behavior. However, it remains questionable whether the observed variation is based on real differences in frequency, or whether it can be explained by multiple dissimilarities in the design of the available studies. Most studies are retrospective analyses. This enlarges the risk of non-representative sampling among different risk groups and geographic areas, limiting the relevancy of the results for other populations. In addition, selection bias due to small numbers or testing at specific indications cannot be ruled out in many studies. Sampling errors can also lead to inclusion of transmission clusters that may overestimate transmission rates.

Differences in the studied population may be particular relevant. Some studies target recently-infected individuals, whereas others only investigate the chronically-infected antiretroviral-naive study population. However, the time that elapsed between infection and sampling may be quite relevant, because transmitted drug resistance may revert or be overgrown in a drug-free environment as present in the new host. Overrepresentation of patients who acquired HIV through a specific route of transmission may also lead to results that can not be generalized to the general HIV-infected population.

Furthermore, as described earlier, the methodology used for susceptibility testing may influence the observed rate of transmitted drug resistance to a great extent. Centers that identify genotypic resistance with a hybridization assay generally detect a higher prevalence of primary resistance, which might be due to the increased capability of this assay to detect minority variants[111,117,118]. However, these results may be biased by the commonly high percentage of hybridization failure with this technique. In addition, the rate of primary phenotypic resistance is generally lower than genotypic resistance. A possible explanation might be that several genotypic substitutions reflecting transmitted drug resistance do not result in decreased susceptibility in isolation.

Finally, there is a lack of uniformity in the definition of transmitted resistance. The currently available consensus mutations lists are based on drug-resistant profiles selected by drug pressure and therefore do not include all indicators of transmission, such as partial revertants. Authors of the various reports interpret the consensus lists differently and frequently make their own adjustments. The use of different definitions can, however, completely shift the prevalence of resistance.

Clinical implications of transmitted drug resistance

Major concerns exist about the clinical implications of transmitted drug resistance. Although it might be expected that the response to antiretroviral therapy is diminished in the case of primary resistance, only limited data assessing clinical outcome are available at present. In a large North American cohort, therapeutic outcome for patients infected with HIV harboring major mutations was not different from patients infected with drug-susceptible viruses[122]. However the majority of patients in this cohort were not prescribed drugs to which the virus they carried exhibited resistance. The small group of patients who did initiate therapy to which the virus exhibited possible resistance at baseline, displayed a relatively inferior virological outcome[122]. In three other studies from the United States and France, the time to viral suppression was significantly prolonged in individuals carrying virus with major phenotypic or genotypic drug resistance at baseline compared to subjects with fully-susceptible virus at baseline[10-12]. Also, the time to virological failure was shorter if primary resistance was present[10]. Additionally, individuals from the ICONA cohort (the Italian Cohort Naive for Antiretrovirals) harboring revertants or atypical mutants at position 215 of RT, had an increased risk of experiencing virological failure compared to those not carrying revertants[13]. These revertants have an increased ability to select the RT T215Y/F mutations that limit the efficacy of thymidine analogues. Of the 13 patients who carried the 215 mutants, 9 experienced virological failure[13].

Some groups report a lower baseline viral load[11,113] or a higher CD4 cell count[12] in antiretroviral-naive individuals carrying primary drug-resistant variants in comparison to individuals with drug-sensitive virus. These differences might reflect variations in duration of infection. Alternative interesting hypotheses suggested by the authors are a decreased viral tropism for tissues involved in T-cell production or a decreased viral replication capacity of the transmitted resistant viruses[11,12,113].

In summary, preliminary data show that transmission of resistance can compromise therapy outcome. Longer follow-up of individuals harboring virus with primary drug resistance is required to reveal whether primary resistance has only deleterious consequences, or whether impaired fitness of transmitted drug resistance may also positively affect the rate of clinical progression. Meanwhile, physicians should consider the possibility of transmission of drug-resistant HIV when initiating therapy or prescribing post-exposure prophylaxis.

Future directions and recommendations

Current guidelines advise considering drug-susceptibility testing when transmission of drug resistance is suspected, or at the initiation of therapy in primary infected patients[134,135]. Modeling carried out by Weinstein, et al.[136] suggest, however, that offering genotypic resistance testing before initiation of therapy is cost-effective in a US health care setting with 4 to 20% prevalence of primary resistance. Obviously, resistance testing and its interpretation have to be validated in clinical studies for the use in recently- and chronically-infected antiretroviral-naive populations.

Currently available algorithms for interpretation of susceptibility tests are based on experience with patients exposed to therapy, and may need modifications for use in antiretroviral-naive patients. Specific transmission algorithms could consider the evolution of resistant viruses after transmission and should include not only partial revertants to wild-type but also other specific patterns that may arise over time from transmitted resistant profiles.

Although transmitted drug-resistant profiles can be retained in the plasma of the new host for a considerable amount of time, mutations may become undetectable in the plasma after a while. However, drug-resistant variants may still be present, archived as proviral HIV-1 DNA in resting cells. Sequencing of proviral HIV-1 DNA instead of regular HIV-1 RNA might elucidate additional information about the diversity of the quasispecies in antiretroviral-naive subjects and its evolution pathways after transmission.

In addition, a more uniform approach, evaluating the frequency of transmission of drug-resistant virus and its clinical implications, is needed. In Europe, 27 countries are participating in the SPREAD-program, which is a European Commission-supported initiative. This program (www.SPREAD-europe.org) is collecting representative quality-controlled genotypic and phenotypic data from more than 4000 HIV-infected individuals. Similar surveillance is undertaken by Health Canada and the CDC in the USA. These programs all collaborate within the WHO program of global surveillance of HIV drug resistance, which is currently being built up.

Conclusion

The prevalence of primary resistant virus varies widely among reports from a number of regions. Currently, it is not clear whether the observed dissimilarities reflect a real difference in the rate of transmission of drug resistance between regions, or if they reflect bias induced by a variety of sampling approaches. Systematic representative global surveillance of the spread of resistance is needed to gain insight into the magnitude of this severe public health problem. Evidently, transmission of drug-resistant HIV is a serious public health problem that might endanger therapeutic options of newly-diagnosed patients. Neither genotypic nor phenotypic tests are validated for use in the antiretroviral-naive population, indicating that research is warranted to elucidate the relevance of these tests in a clinical setting. Design of specific transmission algorithms could support identification and interpretation of genetic profiles that may evolve from resistant viruses after transmission. Ultimately, treatment may have to be adjusted to baseline profiles to avoid the use of ineffective antiretroviral agents and the further evolution of drug resistance.

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