Transmission of drug-resistant HIV-1 in Europe remains limited to single classes

The SPREAD programme*

Background: The spread of drug-resistant HIV-1 might compromise the future success of current first-line regimens.

Objective: To analyse the extent and impact of transmission of drug-resistant HIV-1 variants in Europe.

Design and methods: The European prospective programme (SPREAD) collected demographic, clinical and virological data from 1245 HIV-1-infected individuals in 17 countries diagnosed in 2002–2003. The potential impact of transmitted drug resistance mutations (TDRMs) on therapy response was determined by using genotypic interpretation algorithms.

Results: The overall prevalence of viruses with drug-resistance mutations was 9.1% [96/1050; 95% confidence interval: 7.5–11.1]. The majority (71%) harboured only a single amino acid substitution with limited effect on predicted drug susceptibility. Mutations associated with resistance to nucleoside reverse transcriptase inhibitors were observed most frequently [57/1050 (5.4%)], followed by mutations related to protease inhibitors [32/1050 (3.0%)] and mutations related to non-nucleoside reverse transcriptase inhibitors (NNRTIs) [27/1050 (2.6%)].

In some cases, however, resistance was quite extensive. Four individuals were infected with viruses with reduced susceptibility to all nucleoside reverse transcriptase inhibitors, 3 to all protease inhibitors and 20 to both NNRTIs. Remarkably, in one individual, the resistance pattern was so extensive that none of the available current antiretroviral drugs was predicted to be fully active.

Conclusion: The prevalence of TDRM-HIV is quite prominent (9.1%) but did not increase in comparison with a large retrospective European study. Particularly the presence of single NNRTI mutations may impact the efficacy of the first-line regimens. Continuous prospective monitoring remains indicated to explore the patterns and factors contributing to the transmission of TDRMs as well as the potential clinical consequences.

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Introduction

Combinations of the more recently approved highly active antiretroviral drugs have been shown to be capable of successful long-term suppression of viral replication in the majority of HIV-1-infected patients. Nevertheless, emergence of viral variants with reduced susceptibility to drugs remains an important cause of treatment failure. Loss of control of viral replication during therapy has been associated with an increased risk of transmission of HIV-1 [1,2]. Indeed, the presence of drug-resistant variants in newly diagnosed individuals indicates that a proportion of individuals with treatment failure continue to engage in risk-related behaviour, despite awareness of their HIV-positive status [3].

Although several reports have been published on the spread of drug-resistant viruses in treatment-naive individuals, most studies have limited value, as they are retrospective and based on convenience sampling. To

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date, only a few prospective studies limited to single countries have been published [4,5]. None of the current studies reflects the overall European situation that is characterized by the presence of all relevant transmission groups, men having sex with men, intravenous drug users, heterosexuals and a large proportion of individuals infected in resource-limited countries.

We set up the SPREAD surveillance programme as a prospective monitoring programme to collect the representative data on the spread of resistance among newly diagnosed patients from all risk groups and to estimate the dynamics of the spread of drug-resistant HIV-1 among the approximately 30 000 newly diagnosed individuals each year in Western Europe [6]. Public health institutes and academic centres from 33 countries across Europe participate in the programme (www.SPREAD-europe.org). We present the results of the first round of data collected from 17 countries.

Methods

Data collection

Newly diagnosed HIV-1-infected individuals of age 18 years and older who had never been exposed to antiretroviral drugs were prospectively recruited. Sampling took place in 16 European countries and Israel. A standardized sampling strategy was designed by the epidemiology expert group of the SPREAD programme to ensure representative sampling in all countries. In summary, in countries where more than 80% of all newly diagnosed individuals were expected to be covered by the participating centres (Austria, Denmark, Finland, Greece, Ireland, Israel, Luxembourg, Norway, Serbia Montenegro and Sweden), a random sample from all newly identified individuals was taken. In other countries (Belgium, Germany, Italy, the Netherlands, Poland, Portugal and Spain), stratified sampling weighted for the proportion of newly diagnosed patients among different risk groups and among different geographical areas was performed or the first consecutive number of patients up to a predefined number per geographic region was included [7,8]. Importantly, the sampling strategies were defined in close collaboration with the involved national public health institutes that had access to the latest information on national HIV epidemics. Sample sizes were weighted per country according to the HIV-1 prevalence and based on the calculation that at least 916 individuals were needed to enable detection of an increase in the prevalence over time from 10 to 15% (power of 90%, α of 5%) for future analyses of different collection rounds [7,8].

Epidemiological, clinical and virological data were collected using a comprehensive standardized questionnaire. HIV-RNA plasma levels were collected within 3 months of diagnosis. Newly diagnosed individuals were defined as recently infected if they had documented negative or indeterminate HIV-1 serological results up to 12 months prior to confirmation of diagnosis by western blot. The remaining newly diagnosed individuals were classified as those with undefined duration of infection. Non-European countries were classified as high-prevalence countries if the prevalence of HIV-1 in the population was greater than 1%, as defined by UNAIDS [9].

Patient population

Data from 1245 HIV-1-infected individuals who were newly diagnosed between September 2002 and December 2003 were recorded. Based on the predefined strict entry criteria, 162 individuals could not be included: 24 because of HIV-RNA levels below 1000 copies/ml within 3 months of diagnosis, 130 because no HIV-RNA quantification was performed within 3 months of diagnosis, four because of the absence of confirmatory HIV-1 testing within 6 months and four because of possible therapy exposure. Comparison of the excluded and included set showed a difference in proportion of intravenous drug users (21.0%, 34/162 vs. 8.9%, 96/1083) and the proportion of individuals from high-prevalence countries (9.3%, 15/162 vs. 15.8%, 171/1083).

Procedures

The first available plasma sample obtained within 3 months of HIV-1 diagnosis was used for genotypic resistance analysis. Population-based nucleotide sequence analysis of the HIV-1 *pol* [protease and reverse transcriptase (RT)] gene was successfully performed on 97% (1050/1083) of the samples. For this reason, the denominator for all further analyses was 1050. Sequences were generated by local laboratories using either in-house methods or commercially available kits. All laboratories participated in a continuous blinded quality control programme to verify the quality of the data. Sequence alignment was performed with Clustal X (version 1.81; available at http://bips.u-strasbg.fr/fr/Documentation/ClustalX/) [10].

Transmitted drug resistance mutations (TDRMs) were defined as the presence of at least one of the following mutations in protease: 30N, 46I/L, 48V, 50L/V, 82A/F/T/S, 84A/C/V, 90M; or RT: 41L, 44D, 62V, 65R, 67N, 69D/insert, 70R, 74V, 75I, 77L, 100I, 103N, 106A/M, 108I, 115F, 116Y, 151M, 181C/I, 184I/V, 188C/H/L, 190A/S, 210W, 215Y/F, 215 revertants A/C/D/E/G/H/I/L/N/S/V, 219Q/E, 225H, 230L, 236L. These changes have been identified as related to drug resistance according to the list of mutations of the International AIDS Society USA (IAS-USA) [11]. Other resistance-related mutations in the IAS list which are also known to appear as natural polymorphisms in wild-type HIV-1 (WT-HIV) were excluded as evidence of transmission of resistance. Amino acid changes conferred by the recently approved fusion inhibitor enfuvirtide were also not taken

into account. Mixtures are those of wild-type virus and mutant(s) at a particular codon. Revertants are mutations on position 215 that commonly evolve from the 215Y/F resistance mutations following withdrawal of drug selective pressure due to treatment interruption or transmission.

Viral subtypes were assessed on the basis of the *pol* sequence by using the REGA HIV-1 subtyping tool version 1.0 [12].

We predicted the potential impact of transmitted drug resistance on therapy response by analysing the genotype results using the following interpretation algorithms: the Rega resistance interpretation algorithm (version 6.4.1), the Agence Nationale de Recherches sur le Sida (ANRS) algorithm (version 2005.07; http://hivfrenchresistance.org) and the Stanford drug resistance algorithm (version 4.1.9) [13,14]. The overall predicted susceptibility as estimated by all the three algorithms was very similar (pair-wise κ values exceed 0.6; data not shown). The data in Tables 2 and 3, therefore, refer only to the Rega algorithm.

Statistical methods

The prevalence of transmission of resistance was calculated with a 95% confidence interval (CI) based on the binomial distribution. Categorical data were compared using χ^2-test or Fisher's exact test if appropriate. Continuous data were investigated by means of a *t*-test or the Mann–Whitney *U*-test. Logistic regression analysis was used to examine the association between epidemiological, clinical and virological factors.

Results

A total of 1050 newly diagnosed HIV-1-infected individuals were enrolled of which 22% had laboratory evidence of recent infection (< 1 year). The majority of the individuals (86.3%) contracted HIV-1 through sexual contact. Most them were infected with subtype B virus [690/1050 (65.7%)] (Table 1). Furthermore, 12 non-B subtypes and circulating recombinant forms (CRFs) were identified based on the *pol* gene: A 9.4%, C 8.7%,

Table 1. Comparison of characteristics between patients infected with virus harbouring transmitted drug resistance mutations (TDRMs) and patients infected with wild-type virus.

	Total	TDRM	Wild type	*P* value	OR (95% CI)
Patients [*n* (%)][a]	1050	96 (9)	954 (91)		
Age [years, mean (SD)]	37.1 (11.1)	37.3 (10.9)	37.1 (11.1)	0.847	
Sex (%)[b]					
Male	808 (77)	73 (76)	735 (77)	0.782	0.93 (0.56–1.57)
Female	239 (23)	23 (24)	216 (23)		
Area of origin [*n* (%)][c]					
Western Europe	694 (67)	67 (72)	627 (66)	–	1
Sub-Saharan Africa	169 (16)	13 (14)	156 (17)	0.43	0.78 (0.40–1.50)
Eastern Europe and Central Asia	94 (9)	9 (10)	85 (9)	0.98	0.99 (0.44–2.15)
Other	83 (8)	4 (4)	79 (8)	0.15	0.47 (0.14–1.40)
Route of transmission [*n* (%)][d]					
Homo/bisexual contact	467 (44)	47 (49)	420 (44)	–	1
Heterosexual contact					
Originating from or infected in HPC	193 (18)	10 (10)	183 (19)	0.046	0.49 (0.24–0.99)
Other	246 (23)	30 (31)	216 (23)	0.38	1.24 (0.76–2.02)
Injection drug use	88 (8)	6 (6)	82 (9)	0.35	0.65 (0.27–1.58)
Other	56 (5)	3 (3)	53 (6)	0.26	0.51 (0.12–1.77)
Duration of infection [*n* (%)][d]					
<1 year	235 (22)	25 (26)	210 (22)	0.37	1.25 (0.77–2.02)
Undefined	815 (78)	71 (74)	744 (78)		
CDC stage [*n* (%)][e]					
A and B	807 (86)	77 (87)	730 (86)	0.91	1.04 (0.55–1.97)
C	130 (14)	12 (14)	118 (14)		
CD4 cell count [cells/μl, median (range)][f]	330 (1–1499)	370 (2–1488)	328 (1–1499)	0.49	
HIV-RNA [log copies/ml, mean (SD)][d]	4.83 (0.8)	4.75 (0.8)	4.85 (0.8)	0.22	
Subtype B [*n* (%)][g]	690 (67)	72 (77)	618 (66)	0.029	1.74 (1.05–2.89)
Subtype non-B [*n* (%)][g]	335 (33)	21 (23)	314 (34)		

TDRM, patients infected with HIV-1 with TDRMs; wild type, patients infected with wild-type HIV-1; HPC, high-prevalence country, countries where the prevalence of HIV in the general population exceeds 1%; CDC, Centers for Disease Control. Proportions were compared with *t*-test or Mann–Whitney *U*-test. CI, confidence interval, odds ratio (OR) are calculated with χ^2-test and logistic regression analysis.
[a]Description of patients from whom a baseline HIV genotypic analysis was available.
[b]Data were available for 1047 patients.
[c]Data were available for 1040 patients.
[d]Data were available for 1050 patients.
[e]Data were available for 937 patients.
[f]Data were available for 1013 patients.
[g]Data were available for 1025 patients.

CRF02_AG 4.3%, G 4.2% and less frequently (< 2%) D, F, H, J, CRF03_AB, CRF06_cpx, CRF11_cpx and CRF13_cpx. Seventeen (1.6%) sequences could not be classified. The overall prevalence of TDRMs in HIV-1 strains from newly diagnosed drug naive individuals in Europe was 9.1% (96/1050, 95% CI: 7.5–11.1).

Several factors that might affect the risk of becoming infected with drug-resistant virus were explored (Table 1). The prevalence of TDRM-HIV was not significantly higher in individuals infected for less than 1 year than in individuals with undefined duration of infection [10.6 vs. 8.7%; odds ratio (OR) = 1.3 (95% CI: 0.7–2.0); $P = 0.37$]. TDRMs were identified more frequently in subtype B viruses (10.4%) than in non-B viruses (6.3%) [OR = 1.74 (95% CI: 1.05–2.89); $P = 0.03$]. This difference could be attributed to the lower prevalence of TDRM-HIV in viruses from individuals originally from or known to be infected in countries with a high prevalence of HIV-1 compared with men having sex with men (MSM) [5.2 vs. 10.0%; OR = 0.49 (95% CI: 0.24–0.99); $P = 0.046$]. In fact, when we only considered patients who had been infected within Europe, the prevalence of TDRMs was more comparable among patients infected with subtype B or non-B virus [10.6 vs. 8.1%; OR = 1.3 (0.7–2.7); $P = 0.4$].

Furthermore, we explored the relationship between the presence of TDRM-HIV and infection with other sexually transmitted diseases and sex with anonymous persons and sex for money. None of these factors was significant predictor of infection with drug-resistant HIV-1 using univariate analysis (data not shown). Furthermore, at the time of diagnosis, there were no relevant differences in Centers for Disease Control stages, HIV-RNA levels (4.8 and 4.9 log copies/ml) or CD4 cell counts (370 and 328 cells/μl) between individuals infected with TDRM-HIV or WT-HIV.

Interestingly, among the individuals infected with TDRM-HIV, revertants on position 215 in RT were more frequently detected in individuals with undefined duration of infection [25/71 (35.2%)] than in individuals infected for less than 1 year (3/25, 12.0%) [OR = 3.99 (95% CI: 1.09–14.64); $P = 0.037$]. This result, however, should be confirmed in future studies, as the estimate of

Table 2. Susceptibility of HIV strains with single transmitted drug resistance mutations.

Mutation		Prevalence: Total *n*[a]	Prevalence: As single (%)[b]	Predicted susceptibility to antiretroviral drugs						
NRTI-related				ZDV	D4T	3TC	FTC	ABC	DDI	TDF
Any	–	57	31 (54)	–	–	–	–	–	–	–
41[c]	L	12	2 (17)	I	S	S	S	S	S	S
44	D	4	2 (50)	S	S	S	S	S	S	S
62	V	5	1 (20)	S	S	S	S	S	S	S
70[c]	R	6	1 (17)	I	S	S	S	S	S	S
77	L	4	2 (50)	S	S	S	S	S	S	S
116	Y	3	1 (33)	S	S	S	S	S	S	S
184	V	8	2 (25)	S	S	R	R	S	S	S
215[c]	rev	28	18 (64)	I	S	S	S	S	S	S
219[c]	Q, E	10	2 (20)	I	S	S	S	S	S	S
NNRTI-related				EFV	NVP					
Any	–	27	16 (59)	–	–					
103	N	11	4 (36)	R	R					
108	I	8	7 (88)	S	S					
181	C	3	1 (33)	I	R					
188	H	1	1 (100)	I	R					
190	A	4	2 (50)	I	R					
230	L	1	1 (100)	R	R					
PI-related				APV/r	ATV/r	IDV/r	LPV/r	SQV/r	TPV/r	
Any	–	32	21 (66)	–	–	–	–	–	–	
30	N	4	4 (100)	S	S	S	S	S	S	
46	I, L	15	7 (47)	S	S	S	S	S	S	
82	A, F	8	3 (38)	S	S	S	S	S	S	
90	M	13	7 (54)	S	S	S	S	S	S	

Susceptibility was predicted using the Rega resistance interpretation algorithm (V6.4.1) [13]. NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; rev, revertant: one of the following mutations at position 215 in reverse transcriptase: A/C/D/E/G/H/I/L/N/S/V; ZDV, zidovudine; D4T, stavudine; 3TC, lamivudine; FTC, emtricitabine; ABC, abacavir; DDI, didanosine; TDF, tenofovir; EFV, efavirenz; NVP, nevirapine; APV, amprenavir; ATV, atazanavir; IDV, indinavir; LPV, lopinavir; SQV, saquinavir; TPV, tipranavir; r, ritonavir.

[a]Presence of transmitted drug resistance mutations (TDRMs) at a specific codon.

[b]Number and proportion of TDRMs per codon that occurs as single TDRMs in a sequence.

[c]TAMS, thymidine analogue associated mutations.

the OR was rather imprecise as indicated by the wide limits of the 95% CI. Similarly, mixtures at resistance-related positions were more frequent in individuals with undefined duration of infection [20/71 (28.2%) vs. 5/25 (20%)] [OR = 1.57 (95% CI: 0.52–4.75); $P = 0.43$], but this difference was not statistically significant.

Mutations associated with resistance to nucleoside reverse transcriptase inhibitors (NRTIs) were observed most frequently [57/1050 (5.4%)], followed by mutations related to protease inhibitors (PIs) [32/1050 (3.0%)] and mutations related to non-nucleoside reverse transcriptase inhibitors (NNRTIs) [27/1050 (2.6%)]. The majority [68/96 (71%)] of strains with TDRMs harboured only single drug resistance related amino acid substitutions (Table 2). Consequently, among individuals carrying HIV-1 strains with resistance mutations, a limited proportion [14/96 (14.6%)] harboured mutations related to more than one class of drugs (Table 3).

To evaluate the potential impact of baseline resistance on the efficacy of future therapy, we analysed the RT and protease genotypes using three resistance interpretation algorithms The great majority [967/1050 (92.1%)] of the 1050 HIV-1 strains identified from the newly diagnosed individuals was fully susceptible to all drugs in the three classes. Within the group of viruses with TDRMs, 79.2% of the strains displayed full susceptibility to all NNRTIs, 82.3% to all PIs and 44.8% to all NRTIs (Fig. 1). Moreover, 25% (24/96) of the TDRM-HIV strains were predicted to be fully susceptible to all drugs. These 25 strains contained only single amino acid substitutions, with limited impact on resistance, and therefore were predicted not to have an impact on therapy outcome.

Reduced susceptibility in the remaining TDRM-containing strains was frequently limited to one or only a few drugs within a class (Tables 2 and 3). Some exceptions, however, existed where resistance was quite extensive and affected all drugs of a class (Table 2 NNRTI section, and Table 3). Twenty strains [20/96 (20.8%)] had reduced NNRTI class susceptibility, 14 of these strains were predicted to display high level of resistance to both currently approved NNRTIs. Four strains (4.2%) had reduced susceptibility to all drugs of the NRTI class, one of them was predicted to have high-level resistance to each NRTI. Three (3.1%) strains had reduced susceptibility to all PIs but none was predicted to be highly resistant to all PIs.

Table 3. Characteristics and susceptibility of HIV strains with multiple transmitted drug resistance mutations.

				Number of fully active drugs[a]			
N	NRTI mutations	NNRTI mutations	PI mutations	<1 year	NRTIs (7)	NNRTIs (2)	bPIs (6)
1[b]	41L, 44D, 67N, 74V, 184V, 210W, 215Y	190S	48V, 82S, 90M	Yes	0	0	0
2	41L, 44D, 74VL, 215NHY	103NK, 181C, 190A	46L, 82A		3	0	0
3[b]	41L, 62V, 75I, 215F	100I, 103N	46I, 84V, 90M	Yes	6	0	0
4	41L, 62V, 215Y	181C, 190A	46I, 84V, 90M		2	0	2
5	41L, 215D	–	–		6	2	6
6	41L, 215D	–	–		6	2	6
7	41L, 215D	–	–		6	2	6
8[b]	41L, 210W, 215D	–	–	Yes	5	2	6
9	41L, 210W, 215D	–	–		5	2	6
10	41L, 215N		90M		6	2	6
11	62V, 70RK, 75I, 77L, 116Y, 151M	103N, 108I	46I, 50V, 82A		0	0	1
12	62V, 75I, 77L, 115F, 116Y, 151M	103N	46I, 50V, 82A		0	0	1
13	65RK, 151M	181C	–		0	0	6
14	67N, 219E	–	–		6	2	6
15	67N, 219Q	–	–		6	2	6
16	67N, 219Q	–	–		6	2	6
17	67N, 69D, 70R, 184V, 219Q	–	46L, 82A		1	2	1
18	67N, 69D, 70R, 215F, 219Q	–	–		3	2	6
19	67N, 70R, 184V, 219Q	–	–		1	2	6
20	67N, 70R, 219Q	–	–		4	2	6
21[b]	184V	103N	–	Yes	5	0	6
22	184V	181C	–		5	0	6
23[b]	184V, 215F	–	46I, 90M	Yes	4	2	3
24	215L	–	46L		6	2	6
25	215S	–	90M		6	2	6
26	215S, 219Q	–	–		6	2	6
27	–	103N, 225H	–		7	0	6
28	–	103N, 225P/H	–		7	0	6

Susceptibility was predicted using the Rega resistance interpretation algorithm (V6.4.1) [13].
[a]Resistance-related polymorphisms (not listed) are included in the estimation of drug activity. Nucleoside reverse transcriptase inhibitors (NRTIs): abacavir, didanosine, emtricitabine, lamivudine, stavudine, tenofovir and zidovudine. Non-nucleoside reverse transcriptase inhibitors (NNRTIs): efavirenz and nevirapine. bPIs, boosted protease inhibitors (PIs): atazanavir/r, fosamprenavir/r, indinavir, lopinavir/r, saquinavir/r and tipranavir/r.
[b]These strains were identified in individuals with documented recent infection (<1 year).

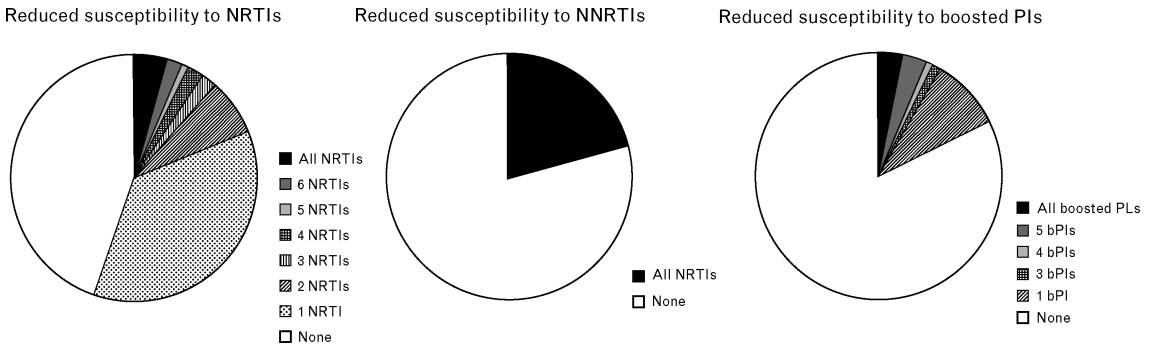

Fig. 1. Estimated susceptibility to antiretroviral drugs of viruses with transmitted drug resistance mutations (TDRMs). Reduced susceptibility of the 96 viruses with TDRMs to the different drug classes was calculated using the Rega algorithm. Nucleoside reverse transcriptase inhibitors (NRTIs): abacavir, didanosine, emtricitabine, lamivudine, stavudine, tenofovir and zudividine. Non-nucleoside reverse transcriptase inhibitors (NNRTIs): efavirenz and nevirapine. Boosted protease inhibitors (PIs): amprenavir/r, atazanavir/r, idinavir/r, lopinavir/r, saquinavir/r and tipranavir/r.

Remarkably, in one individual, the resistance pattern was so extensive that none of the currently available antiretroviral drugs was predicted to be fully active. Loss of susceptibility was most extensive for the NRTI zidovudine, with only 50% (48/96) of the strains with TDRMs predicted to be fully susceptible, 27.1% displaying intermediate resistance and 22.9% high-level resistance. In contrast, the least affected drug was the PI tipranavir, for which 96.9% of all strains harbouring TDRMs were predicted to be fully susceptible.

Discussion

The SPREAD programme is the first large, prospective and sufficiently powered multinational European study performing a well-controlled assessment of the prevalence of transmitted drug resistance. This study prospectively studied transmission of resistant virus in a representative population of over 1000 newly diagnosed HIV-1-infected individuals, consisting of 235 individuals in whom a recent seroconversion was documented.

Our study shows that the prevalence of viruses with transmitted drug resistance mutations in newly diagnosed individuals is around 9% and that in the majority of individuals only single drug resistance-related mutations are detected.

For two reasons, it cannot be excluded that we underestimate the transmission of drug-resistant mutations. First, we used population sequencing for detection of resistance-related mutations and it is possible that mutant populations comprising a minority of the viral population remained undetected [15,16]. Second, we assessed prevalence of TDRMs among newly HIV-1-diagnosed individuals. The population of newly diagnosed individuals in our study represents a range from acutely infected individuals to those with a chronic HIV-1 infection. Although persistence of drug-resistant mutant viruses in the absence of selection pressure of therapy frequently has been reported [17–20], it is possible that in the period between infection and diagnosis, reversion to wild-type and/or outgrowth of minority wild-type species may result in disappearance of resistant viruses from plasma. Indeed, we observed a trend towards a higher prevalence of resistance-related mutants but a lower prevalence of mixture and revertants in individuals with documented recent infection. These results indicate that transmitted mutations can revert over time in the absence of therapy.

In the retrospective Child and Adolescent Trial for Cardiovascular Health (CATCH) study, which collected samples from 1996 to 2002 before the start of the prospective SPREAD programme, we reported earlier a higher prevalence of TDRMs in subtype B viruses than in non-B viruses [21]. Also, in this prospective study, we identified more frequently TDRMs in subtype B viruses compared with non-B viruses (10.4 vs. 6.3%). Using the epidemiological data collected in the SPREAD programme, we established that a vast majority of patients from or known to be infected in countries with a high prevalence of HIV-1 carried predominantly non-B virus, the wild-type HIV-1. In contrast, the prevalence of TDRM-HIV was equally distributed among B and non-B strains carried by patients who had been infected in Europe. This indicates that the overall difference observed is not attributed to specific viral characteristics of non-B strains but rather to the lack of exposure to drugs in the high-prevalence countries [22].

With the current lack of prospective data collected over a prolonged period, making definite statements regarding the patterns and prevalence of TDRM changes over time is still difficult. In the retrospective CATCH study, however, a prevalence of TDRMs of 10% was reported from surveillance data from several European countries between 1996 and 2002 [21]. In Fig. 2, the incidence of

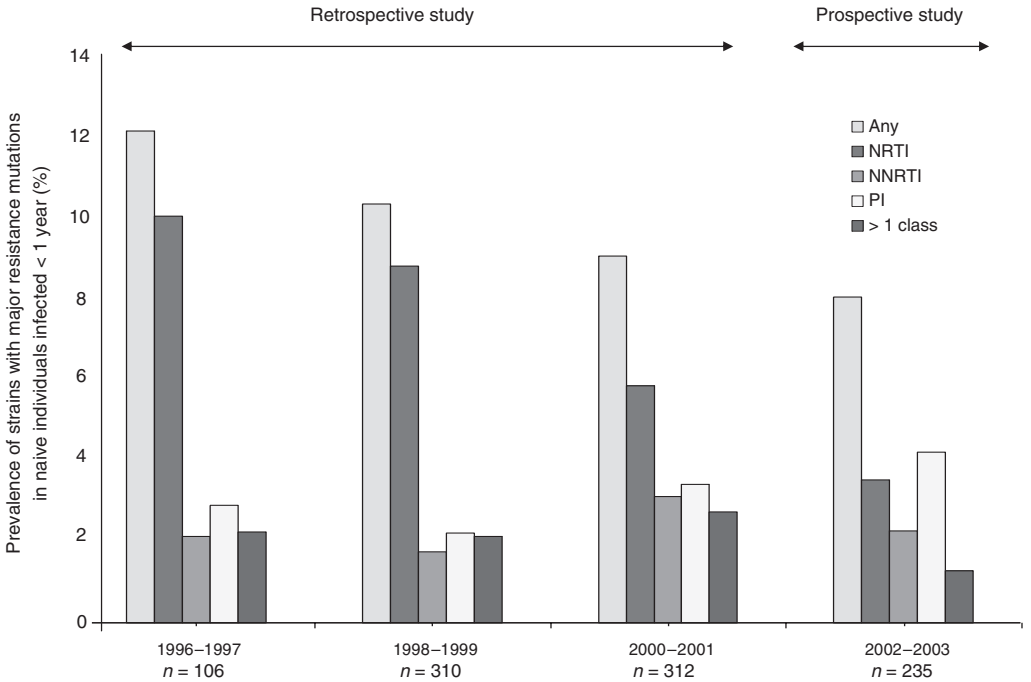

Fig. 2. Transmitted drug resistance over time. NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor.

TDRMs in recently infected patients (<1 year) from the retrospective CATCH study (data taken from 1996 to 2001) and the prospective SPREAD study (data from 2002 to 2003) is shown. Despite the limitations of comparing two datasets with different sampling strategies, it is still of interest to see that the proportion of recently infected individuals diagnosed with strains harbouring TDRMs does not continue to rise but seems to decrease over time in Europe [23] (Fig. 2).

The results of SPREAD are generally consistent with the results of several other studies that recruited various risk groups from individual high-income countries, with prevalences ranging from 0 to 17% in Europe [4]. Some observational and retrospective studies in the US and UK observed an increased prevalence of TDRMs in recently infected individuals over time to more than 20% in recent years [24–27]. These studies, however, had a different design with an inclusion of predominantly MSM in urban areas exposed to highly antiretroviral drugs. In contrast, a sentinel study describing data from 1083 individuals collected during 1998–2000 in several cities in the US using a consecutive sampling approach observed a prevalence of TDRMs of 7.4% among chronically infected patients and 12% among recently infected patients [5]. The prevalence of TDRMs in this study was significantly higher in MSM (11% in chronically and 15% in recently infected) than in heterosexual infected individuals. In our study, the prevalence of TDRMs was comparable and not significantly higher in MSM as compared with heterosexual individuals infected in Europe (11 vs. 10%). The prevalence of TDRMs in MSM, however, was significantly higher compared with patients coming from outside Europe (5.5%). These results indicate the importance of collection of epidemiological data from all transmission groups for surveillance of TDRMs.

Insights into the dynamics of transmission of drug-resistant viruses can be obtained by analysing the patterns of transmitted mutations in more detail. The predominance of single thymidine-associated mutations (TAMSs) and 215 revertants can be explained by a combination of factors. TAMSs are selected by the thymidine analogues (zidovudine and stavudine); therefore, transmission of viruses with solitary TAMS most likely reflects a predominant circulation of these viruses at points in time (e.g. late 1980s and early 1990s), when there was extensive use of non-suppressive mono and dual therapies with thymidine analogues. Introduction of highly active antiretroviral therapy in the mid-1990s caused a more equal distribution of resistance among the three classes in patients with treatment failure. As a result, more recent transmissions patterns show a relative decrease in the proportion of NRTI resistance (Fig. 2).

Given the fact that the majority of the HIV-1 strains harboured only a single mutation, the effect on the response to future therapy may be limited. Indeed, a quarter of the isolated viruses harbouring TDRMs were predicted to be fully susceptible to all currently approved antiretroviral drugs. Nevertheless, identification of these resistance-related mutations is essential as they give insight into the transmission patterns of viruses that have been exposed to therapy in previous hosts.

The predicted effect of other single TDRMs on therapy outcome depends on the specific class of antiretroviral drugs. The presence of most single TAMS is not predicted to affect initial therapy response dramatically, especially given that NRTIs are in general combined with a NNRTI or PI. Then again, the presence of baseline resistance mutations can decrease the genetic barrier, reducing the number of mutations necessary for loss of susceptibility. For instance, the presence of baseline 215 revertants has been reported to be associated with a higher risk of virological failure on regimens containing a thymidine analogue [28].

The effect of single PI mutations on first-line therapy is expected to be limited in the era of boosted PIs because multiple mutations have to be generated before therapy fails [29].

In contrast, most solitary NNRTI mutations are sufficient for a complete loss of activity of the first-generation NNRTIs (efavirenz and nevirapine) [30]. Treatment guidelines advise to initiate first-line therapy with a boosted PI or a NNRTI accompanied by a NRTI backbone. As solitary mutations may dramatically effect the susceptibility of NNRTIs, we support the recommendations for baseline resistance testing for newly diagnosed individuals and, if necessary, subsequent customizing of initial therapy. Furthermore, minority strains with transmitted drug resistance related mutations might not always be detected by conventional resistance testing [15,16]. A single mutation or a revertant might be an indication that more extensive resistance has been transmitted. Therefore, in cases of infection with HIV-1 carrying a single transmitted drug resistance mutation, the initiation of antiretroviral combination regimens with a high genetic barrier should be considered. In addition, treatment response should be closely monitored and resistance testing should be urgently considered if treatment failure is suspected.

In conclusion, we have shown that the prevalence of viruses with transmitted drug resistance mutations in newly diagnosed individuals in Western Europe and Israel is approximately 9% and that in the majority of cases detection is limited to single drug resistance-related mutations.

Continuous monitoring remains indicated to determine the patterns, rates and factors contributing to the transmission of TDRMs, as well as the potential clinical consequences.

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