Impact of highly active antiretroviral therapy on the molecular epidemiology of newly diagnosed HIV infections

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Objective: To evaluate HIV-1 transmission trends and the impact of highly active antiretroviral therapy (HAART) on newly diagnosed HIV infections in Geneva, Switzerland.

Design: Retrospective molecular epidemiology analysis of all newly HIV-diagnosed individuals between 2008 and 2010.

Methods: Phylogenetic analyses were performed using *pol* sequences of 780 newly HIV-1 diagnosed individuals between 2000 and 2010 (mandatory reporting) and 1058 individuals diagnosed before 2000. All clusters (bootstrap value >98%) including individuals diagnosed in 2008–2010 were analyzed. Recent HIV infections (<1 year) were determined by documented seroconversion and/or fraction of ambiguous nucleotides. Median viral load and HAART coverage during the study period were obtained from patients included in the Swiss HIV Cohort Study (SHCS).

Results: Among 142 newly diagnosed individuals during 2008–2010, 49% had a recent infection and 42% were included in transmission clusters. Among the latter, two-thirds were included in new clusters and one-third expanded previously known clusters. MSM carrying resistant strains were more frequently included in clusters. Only 1.8% of individuals diagnosed before 2000 and 10.8% diagnosed during 2000–2008 were included in clusters involving individuals diagnosed between 2008 and 2010. During 2008–2010, the median population viral load of SHCS-enrolled individuals was significantly lower for individuals diagnosed before 2000 than for those diagnosed during 2000–2008 and 2008–2010 and HAART coverage significantly higher.

Conclusions: MSM with recent HIV infection are a significant source of onward transmission. Individuals diagnosed before 2000 were only exceptionally related to newly diagnosed infections between 2008 and 2010. Prevention campaigns need to be focused on improving diagnosis for recently infected individuals.

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Introduction

The evidence for increased complications in untreated HIV-positive individuals [1,2], even at relatively high CD4 T-cell counts, has led to an expansion of highly active antiretroviral therapy (HAART) indications and, consequently, to an increase in the number of treated patients worldwide. A growing body of evidence also

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suggests an important role of HAART as a tool to prevent HIV transmission with public health implications. Transmission of HIV during sexual intercourse [3], or from mother to child [4] depends largely on the viral load. As HAART reduces the viral load in blood and genital secretions, it decreases the infectiousness of treated patients. Mathematical models, large ecological studies [5–7] and clinical trials performed in serodiscordant couples [8,9], have recently confirmed the key role of antiretrovirals to prevent HIV spread. The possible epidemiological impact of 'Treatment as Prevention' (TasP) has become a strong argument to increase access to treatment in resource-limited areas and to push HAART expansion in developed countries [10,11]. However, some phylogenetic studies have suggested that individuals with recent HIV infection may represent an important source for HIV spread, which might limit the efficacy of TasP [12–14]. Recently infected individuals frequently have a high viral load and may be unaware of their HIV-infection.

The County of Geneva in Switzerland is a small geographic area with around 450 000 inhabitants and a high proportion of foreigners. According to the Swiss Federal Office of Public Health, 650 new HIV infections were diagnosed in Switzerland in 2010 with around 65 in Geneva. The molecular epidemiology of HIV infection in Geneva County has been closely followed since the 1990s [14]. The laboratory of virology at the University of Geneva Hospitals confirms all newly diagnosed HIV infections and performs all resistance testing for the County. More than 95% of cases diagnosed in Geneva are notified to the Swiss Federal Office of Public Health. The Swiss HIV Cohort Study (SHCS) is a prospective cohort that enrols approximately 75% of HIV-diagnosed individuals in Switzerland and Geneva and is highly representative of the characteristics of the overall HIV-infected population in Switzerland [15]. Similar to other resource-rich countries, the number of treated patients has increased in recent years following guidelines that recommend treatment earlier in the course of HIV infection. We performed this study to evaluate HIV-1 transmission trends and the impact of HAART on transmission clusters, including individuals newly HIV-diagnosed between 2008 and 2010 in Geneva.

Methods

Study population

On the basis of the mandatory reporting of all newly diagnosed cases of HIV infection in Switzerland, the sequences of all individuals resident in the Geneva County with a newly HIV infection diagnosed between January 2000 and April 2010 were included in the study. All individuals resident in Geneva with genotypic resistance testing performed during the same period and not included in the newly diagnosed dataset were selected from our centralized database (IDNS™; SmartGene, Zug, Switzerland). All were infected before 2000 and were followed either at our university medical center or by private practitioners. For individuals with multiple resistance testing, only the first sequence (2000–2008) was considered. All sequences were anonymized prior to analyses.

Information on risk factors, sex, age and ethnicity were obtained from mandatory reporting of all newly HIV-diagnosed individuals resident in Geneva County between January 2000 and April 2010.

Transmission of drug resistance

Population-based sequence analysis of the reverse transcriptase and protease regions was performed on an Applied Biosystems sequence as previously described [16]. Transmission of drug-resistant (TDR) virus was evaluated using the list of mutations for TDR surveillance [17].

Phylogenetic analyses

Sequences of 780 newly HIV-1 diagnosed individuals between 2000 and 2010 and of 1058 individuals diagnosed before 2000 were included. Phylogenetic analyses were performed by maximum likelihood trees using *pol* sequences (protease region-reverse transcriptase, 895 bp). Transmission clusters were defined as related groups with bootstrap values at least 980/1000. When a small cluster was embedded in a larger one, only the latter was considered. Cluster directly below the root, or two levels below were ignored.

Clusters including at least one newly diagnosed individual between May 2008 and April 2010 were analyzed and classified as either 'new cluster' or 'expansion of previously known cluster' if this cluster was already known in the previous analysis including newly HIV-1 diagnosed individuals between 2000 and April 2008 [14]. Individuals were classified according to their time of HIV diagnosis: before 2000; between 2000 and 2008 (according to the selection criteria of our previous study [14]); and between 2008 and 2010. Recent HIV infections (defined as less than 1 year) were identified by documented seroconversion, patterns of immunoblot tests [18] and/or fraction of ambiguous nucleotides less than 0.5%. Position with more than one nucleotide identified was defined as 'ambiguous nucleotide' when the less frequent exceeded 20% [19]. To avoid double testing, patients tested in anonymous testing centers and included in a cluster were excluded, if they shared the same year or birth.

Patients on antiretroviral therapy and population HIV-1 viral load

The percentage of patients on HAART, median population HIV-1 viral load, and the proportion of

patients with an undetectable HIV-1 viral load in Geneva were evaluated from the patients included in the SHCS. To calculate the median viral load during the study period, the viral load measure for each patient was considered to obtain the individual median viral load. The median viral load of all patients was then calculated for the groups of individuals diagnosed before 2000, between 2000 and 2008, and between 2008 and 2010. For the purpose of the statistical analyses, HIV-1 RNA below the limit of detection (less than 20 or less than 40 copies/ml according to the period of the study) was considered as 10 copies/ml. For each of the above-mentioned groups, six, six and five determinations of viral load, respectively, were available during the 2-year study period (2008–2010).

Statistical analyses

To evaluate factors associated with inclusion in transmission clusters, univariable and multivariable logistic regression models were performed with an alpha threshold of 5%. Variables included were age, ethnicity, sex, category of risk for transmission, HIV-1 RNA (per log copies/ml), CD4 cell counts (per 100 cells/μl), recent HIV infection (less than 1 year), TDR, HIV-1 subtypes (B or other). For all patients enrolled in the SHCS and diagnosed before 2000, between 2000–2008 and during the study period (May 2008 to April 2010), we computed the proportion of patients on HAART and median age at the end of the study period, median viral load and the proportion with an undetectable viral load (less than 40 HIV-1 RNA copies/ml) or below 1000 copies/ml during the study period. The chi-square test with a threshold of 5% was used to test the difference for qualitative variables between the three periods of HIV diagnosis, and the Kruskal–Wallis test was used to test the difference for quantitative variables with a threshold of 5%. Statistical analysis was performed using STATA software, version 12.0 (StataCorp, College Station, Texas, USA).

Results

During the study period (May 2008 to April 2010), 142 new HIV-1 infections were diagnosed in Geneva County. The demographics and characteristics of HIV infection of the population are shown in Table 1. Approximately two-thirds of individuals were men, and the most frequent risk factor was heterosexual unprotected sex. Approximately one-third of individuals were European and one-third of African origin. In around 20% of cases, the geographic origin and the risk factor were not known as individuals were tested in anonymous test sites. Almost half of the newly diagnosed individuals were infected with non-B HIV-1 subtypes and circulating recombinant forms (CRFs) (19.6% of CRF-02, 9.2% of C subtype, 4.2% of A, 2.8% of CRF-01 and F among the most frequent) and 49% were diagnosed less than 1 year after infection.

Table 1. Demographic and HIV characteristics of 142 newly diagnosed HIV individuals in 2008–2010.

Demography	*N* (%)
Men	90 (64)
Age (years)[a]	39 (30–45)
Risk factors	
Men who have sex with men	47 (33)
Heterosexual	60 (42)
Injecting drug user	6 (4)
Other	4 (3)
Unknown[b]	25 (18)
Continent of origin	
Europe	51 (36)
Africa	44 (31)
South America	10 (7)
Asia	6 (4)
Unknown[b]	31 (22)
HIV infection	
HIV RNA (log copies/ml)[a]	4.66 (4.08–5.36)
$CD4^+$ (cells/μl)[a]	262 (134–394)
Subtypes non-B	63 (44)
Recent infection (<1 year)[c]	70 (49)
Drug resistance	15 (10.6)
NRTI	11 (7.7)
NNRTI	5 (3.5)
PI	7 (4.9)
>1 class	6 (4.2)

NNRTI, nonnucleoside reverse transcriptase inhibitor; NRTI, nucleoside/nucleotide reverse transcriptase inhibitor; PI, protease inhibitor.
[a]Median, interquartile range.
[b]Individuals diagnosed in anonymous testing centers.
[c]Defined by documented seroconversion and/or fraction of ambiguous nucleotides (<0.5%) [18,19].

Transmission of HIV-1 drug-resistant strains was identified in 15 (10.6%) newly diagnosed individuals.

Viral sequences of 1058 individuals diagnosed before 2000, 638 individuals diagnosed between 2000 and 2008, and the 142 individuals diagnosed in 2008–2010 were used to build the phylogenetic tree, which allowed us to identify 214 clusters of infection including two to 13 individuals. Of the 214 clusters, 35 clusters (148 individuals) included at least one individual diagnosed between 2008 and 2010. Overall, 60 of 142 individuals (42%) diagnosed between 2008 and 2010 were included in transmission clusters. Parts of the phylogenetic tree illustrating transmission clusters can be seen in Fig. 1.

Table 2 presents the results of the univariate and multivariate logistic regression model assessing the risk factors of belonging to transmission cluster. Being part of transmission cluster was significantly associated with recent infection, through homosexual contact, male sex, Caucasian origin, infection with subtype B and drug-resistant virus. Multivariate analyses indicated homosexual transmission and detection of drug-resistant virus as the most potent predictors for being part of transmission clusters. The final model containing all predictors of interest was statistically significant (chi-squared = 23.7; $P < 0.0001$).

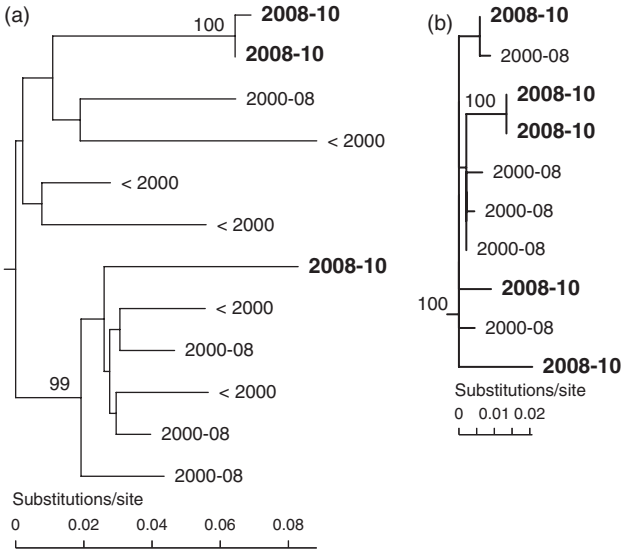

Fig. 1. Parts of the phylogenetic tree illustrating transmission clusters. Maximum likelihood tree was performed using sequences from 1058 individuals diagnosed before 2000, 638 individuals diagnosed between 2000 and 2008, and the 142 individuals diagnosed in 2008–2010. Clusters including at least one newly diagnosed individual between May 2008 and April 2010 (2008–2010) were analyzed and classified as either 'new cluster' or 'expansion of previously known cluster' if this cluster was already known in the previous analysis including newly HIV-1 diagnosed individuals between 2000 and April 2008 (2000–08) [14]. Individuals diagnosed between 2008 and 2010 and in transmission clusters (bootstrap values $\geq$ 98%) shown in bold. (a) shows a new cluster among two individuals diagnosed between 2008 and 2010 (top), individuals not included in transmission clusters (middle) and the expansion of a previously known transmission cluster (bottom). (b) shows the expansion of a previously known cluster of multidrug resistant virus, carrying the reverse transcriptase 69 insertion.

Among the 15 individuals with drug-resistance mutations, 12 (80%) were included in clusters. Five individuals harbored reverse transcriptase resistance mutations 69insertion, 70R and protease resistance mutations 71 V, 77I, 90 M; two individuals harbored reverse transcriptase 41L and 215E. The other individuals harbored reverse transcriptase resistance mutations 215S or 215A or 41L, 210W, 215C or 103N or 138A.

To characterize the dynamics of transmission clusters, we analyzed whether newly diagnosed individuals in 2008–2010 expanded previously known clusters [14] or were part of a new cluster. Among the 60 newly diagnosed individuals in 2008–2010, 39 (65%) were included in one of the 20 newly identified clusters, and 21 (35%) expanded one of 15 previously known clusters (Fig. 2a). Non-B subtypes (including CRFs) were identified in 23.3% of individuals included in transmission clusters (14.3% for previously known clusters and 28.2% for new clusters) and in 59.8% of nonclustered newly diagnosed infections.

We analyzed next the period of diagnosis of the individuals who were in clusters with the newly diagnosed individuals in 2008–2010. Among the individuals included in new clusters, 41% were in clusters exclusively with other individuals diagnosed in 2008–2010, all presenting a recent infection. Fifty-one percent were in clusters with individuals diagnosed between 2000 and 2008, and only 8% were in clusters including exclusively individuals diagnosed before 2000. Among individuals included in previously known clusters, 76% were in clusters containing only individuals diagnosed between 2000 and 2008 and 24% were in expanded clusters including both individuals diagnosed before 2000 and between 2000 and 2008. None of the expanded clusters included exclusively individuals diagnosed before 2000 (Fig. 2b and c).

Figure 3a presents the proportion of individuals included in clusters with newly diagnosed individuals in 2008–2010 according to their period of diagnosis. Individuals infected before 2000 were less frequently included in clusters with individuals diagnosed in 2008–2010 (1.8%; 19/1059) than those diagnosed between 2000 and 2008 (10.5%; 69/638) and during the study period (42.3%; 60/142). Individuals with a recent infection at the time of diagnosis were overrepresented in clusters including individuals diagnosed in 2008–2010 (66%; 98/148) compared with those with chronic infections (34%; 50/148).

Population viral load and HAART coverage in Geneva County

During the study period, individuals enrolled in the SHCS and diagnosed before 2000 had wider HAART coverage and a higher proportion of undetectable viral load than individuals diagnosed between 2000 and 2008 and those diagnosed, between 2008 and 2010 (84 vs. 75%, $P < 0.0001$ and 50 vs. 42%, $P = 0.005$; respectively) and than those diagnosed between 2008 and 2010 (51%, $P < 0.0001$ and 7%, $P < 0.0001$; respectively) (Fig. 3b). The median viral load during the whole study period was significantly higher for individuals diagnosed between 2008 and 2010 [median (SD), 3.13 (1.19) log HIV-1 RNA copies/ml] when compared with individuals diagnosed between 2000 and 2008 [1.74 (1.05) HIV-1 RNA copies/ml and before 2000 (1.54 (0.86) log HIV-1 RNA copies/ml ($P = 0.0001$ for all periods]. The median age was 48 years for individuals diagnosed before 2000, 41 years for those diagnosed between 2000 and 2008, and 36 years for those diagnosed between 2008 and 2010 ($P = 0.0001$ for all periods).

Discussion

In this epidemiologic and molecular analysis, we found that almost half of all newly diagnosed cases belonged to

Table 2. Factors associated with inclusion in clusters for individuals newly diagnosed in 2008–2010.

	In clusters ($N = 60$)	Not in clusters ($N = 82$)	Univariable analysis		Multivariable analysis	
			Odds ratio (95% CI)	*P*	Odds ratio (95% CI)	*P*
Age (years)[a]	36 (29–43)	39 (30–46)	0.98 (0.96–1.01)	0.428		
Male	84%	50%	5.44 (2.37–12.51)	0.000		
Ethnicity						
Black	13%	44%	1			
Caucasian	59%	21%	9.26 (3.54–24.20)	0.000		
Others	28%	35%	2.63 (0.99–6.97)	0.051		
Risk factor for transmission						
Heterosexual	35%	47%	1		1	
MSM	50%	21%	3.27 (1.47–7.27)	0.004	2.64 (1.11–6.26)	0.027
Others	15%	32%	0.64 (0.89–0.99)	0.349	0.54 (0.20–1.46)	0.231
HIV RNA (per log copies/ml)[a]	4.69 (4.26–5.32)	4.65 (3.88–5.36)	1.03 (0.71–1.48)	0.867		
CD4 count (per 100 cells/μl)[a]	234 (84–375)	275 (134–414)	0.99 (0.99–1.00)	0.793		
Recent infection (<1 year)	60%	40%	2.22 (1.12–4.39)	0.021	1.39 (0.64–3.04)	0.397
Drug-resistant virus	22%	2%	5.39 (1.66–17.51)	0.042	5.69 (1.60–20.16)	0.011
Subtype B infection	77%	40%	4.87 (2.31–10.26)	0.000		

CI, confidence interval.
[a]Median (interquartile range).

transmission clusters. Recent HIV infections were a significant source of HIV spread. By contrast, HIV individuals diagnosed before 2000 were rarely the source of new infections after 2008. Mathematical models, prevention of mother-to-child transmission [4], ecological studies [5–7] and trials in serodiscordant couples [8,9] have consistently shown the preventive role of antiretroviral drugs. This growing evidence has expanded the indications for treatment regardless of the CD4 T-cell count not only for individual benefit, but

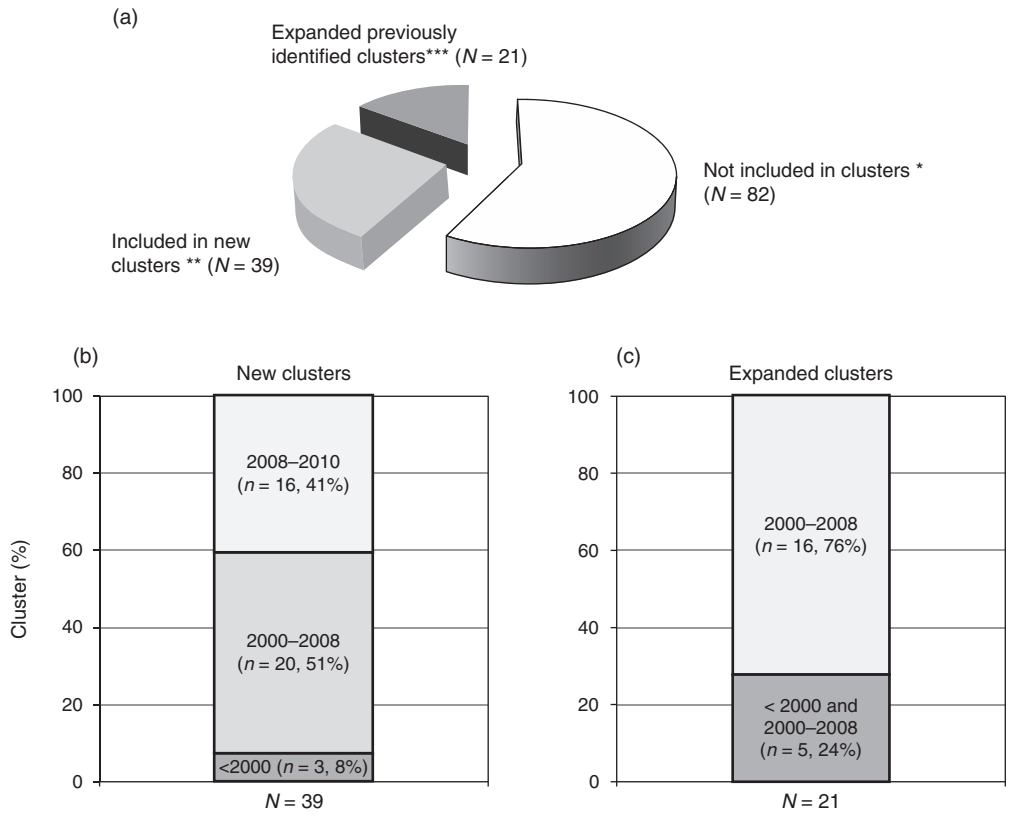

Fig. 2. Dynamics of transmission clusters. Proportion of newly diagnosed individuals in 2008–2010 included or not in transmission clusters (a). Period of diagnosis of individuals in cluster with the newly diagnosed individuals in 2008–2010 and included in new clusters (b) or expanding previously identified clusters (c). * Not included in clusters: no related infections identified for these sequences. ** Clusters of transmission not previously known in the Geneva County. ***'Previously identified clusters' refers to those clusters of transmission already reported in [14].

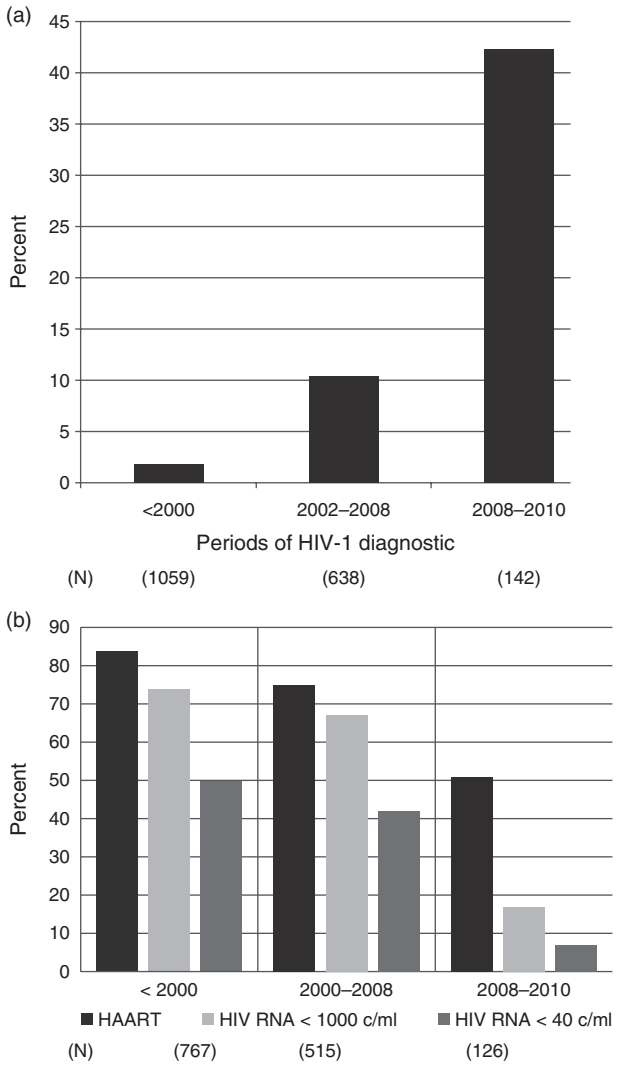

Fig. 3. Impact of highly active antiretroviral therapy on transmission clusters. (a). Proportion of individuals included in clusters of transmission with newly diagnosed individuals in 2008–2010 according to the period of diagnosis. Among the 1059 individuals infected before 2000 (<2000), 19 (1.8%) individuals were part of transmission clusters including individuals diagnosed in 2008–2010, whereas 69/638 (10.5%) individuals diagnosed between 2000–2008 and 60/142 (42.3%) individuals diagnosed between 2008 and 2010 were in transmission clusters. (b). Proportion of individuals on HAART, with HIV RNA below 1000 copies/ml and below 40 copies/ml among the participants of the Swiss HIV Cohort Study during the study period (May 2008 and April 2010). Among the participants of the Swiss HIV Cohort Study, 767 individuals diagnosed before 2000 (<2000), 515 individuals diagnosed between 2000–10 and 126 individuals diagnosed between 2008 and 2010 with available HIV-1 RNA levels during the study period were included in this analysis.

also to prevent new infections. However, a number of phylogenetic studies have suggested the important impact of recent infections on HIV spread [12–14].

Our study adds to the body of evidence for both concepts. By tracing the related infections of individuals diagnosed during the study period, we show that the epidemic is only marginally fed by individuals diagnosed over a decade ago (before 2000). This may be related to their lower viral load as a consequence of HAART coverage. Other factors could also contribute to this finding, such as the older age of this group, with less risky behavior for HIV transmission. By contrast, recently infected, untreated individuals are a frequent source of new infections in Geneva.

In our study, inclusion in clusters was more frequent for MSM patients of European origin carrying a subtype B virus and with recent infection as shown in other European countries [20]. Heterosexual individuals of African origin were infected most probably several years ago in their countries of origin. Thus, we found that only 18% of newly diagnosed individuals of African origin were included in transmission clusters. The impact of moving population coming from abroad is also shown by the subtype distribution among individuals included or not included in clusters, as the non-B subtypes rises to almost 60% for those not included in clusters compared with 23.3% for those included in clusters of transmission. Another recently published Swiss study has shown that a subtype B epidemic is not self-sustaining in a heterosexual population and this subtype is rarely evolving in clusters among heterosexuals and injecting drug users [21].

Through the continuous surveillance of HIV transmission trends in Geneva County [14], we were able to identify those individuals who were part of a new transmission cluster and those who were expanding already known clusters (see Fig. 1). Two-thirds of individuals in transmission clusters were included in new clusters. Among those included in new clusters and related exclusively to other individuals diagnosed between 2008 and 2010, there were 100% of recent infections, thus, highlighting the impact of recently infected individuals in transmission. However, one-third expanded already known clusters, suggesting that chronically infected patients contribute also to HIV spread.

TDR was around 10%, which is comparable to our previous report [14] and to large muticohort studies [22]. However, as previously reported by us and other groups [14,23], inclusion in clusters was more frequent for individuals with drug-resistant virus than nonresistant strains. This may not represent the expression of an increased transmissibility of the resistant strain, but rather a marker of a risk category, as most individuals included in transmission clusters of resistant viruses were MSM. Among them, the largest cluster involving resistant strains included five MSM (all with recent infection) carrying a virus with the reverse transcriptase 69 insertion, conferring cross-resistance to all nucleosides/nucleotides (Fig. 3b). As expected, all drug-resistant mutations

detected in these clusters were mutations not reported to impact significantly replicative fitness [24]. The mutation M184 V, mostly associated with reduced fitness was never detected in our population of newly diagnosed individuals.

Our study is original for several reasons. It is one of the few studies [12] combining molecular epidemiology with the demography of newly diagnosed individuals and showing the dynamics of transmission clusters over the time in a longitudinal manner. According to mandatory reporting, the population of newly diagnosed individuals included in our study represents about 95% of all individuals diagnosed between 2000 and 2010. Moreover, SHCS enrolls most individuals diagnosed in Geneva County. This ensures the high representativeness of the presented data. The 98% chosen bootstrap value ensures the strict criteria for related infections. However, this study also has several limitations. For the 51% of nonrecent infections, it is not possible to establish a correlation between transmission and community viral load since the timing of infection is unknown. Thus, the median viral load during 2008–2010 cannot be correlated only with transmissibility, except probably for recent infections. Indeed, this is also the main limitation of most ecological studies as cause-effect cannot be proved and results remain descriptive. The same limitation applies to individuals acquiring the infection elsewhere and diagnosed in Geneva and where no relation can be supposed. A recent molecular epidemiology study focusing on non-B infections demonstrates that less than a quarter of all non-B infections diagnosed in Switzerland could possibly be prevented by local interventions [25].

In conclusion, the ongoing HIV epidemic in Geneva is mostly fed by individuals with recent infection, notably those occurring among MSM are frequently included in transmission clusters. Prevention campaigns need to be focused on improving diagnosis for recently infected individuals who represent an important source of HIV transmission. The group with higher HAART coverage (patients diagnosed before 2000) is rarely involved in new HIV infections and confirms that expanded access to HAART may impact on epidemiological patterns. Repeated waves of testing for those groups at the highest risk should be encouraged, particularly for MSM. If measures to increase testing and earlier diagnosis are not implemented, a TasP strategy, even applied on a large scale, will lose part of its potential to contain this public health problem. Similar studies should be encouraged in other regions to adapt prevention politics to the local epidemiology.

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S.Y. and J.A. designed and conducted the study and drafted the manuscript. T.J. performed phylogenetic analyses under the supervision of E.Z. C.D., S.Y. and J.A. analysed the data. A.C., B.H. and L.K. designed the study. All authors have reviewed the latest version of the manuscript and have approved its content.

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Conflicts of interest

There are no conflicts of interest.

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