Journal of Infectious Diseases Advance Access published January 9, 2015

Assessing the paradox between transmitted and acquired HIV-1 drug resistance in the Swiss HIV Cohort Study from 1998 to 2012

Wan-Lin Yang[1], Roger Kouyos[1], Alexandra U Scherrer[1], Jürg Böni[2], Cyril Shah[2], Sabine Yerly[3], Thomas Klimkait[4], Vincent Aubert[5], Hansjakob Furrer[6], Manuel Battegay[7], Matthias Cavassini[8], Enos Bernasconi[9], Pietro Vernazza[10], Leonhard Held[11], Bruno Ledergerber[1], Huldrych F. Günthard[1], and the Swiss HIV Cohort Study (SHCS)

[1]Division of Infectious Diseases and Hospital Epidemiology, University Hospital Zurich, University of Zurich, Zurich, Switzerland

[2]Swiss National Center for Retroviruses, Institute of Medical Virology, University of Zurich, Zurich, Switzerland

[3]Laboratory of Virology, Division of Infectious Diseases, Geneva University Hospital, Geneva, Switzerland

[4]Department of Biomedicine-Petersplatz, University of Basel, Basel, Switzerland

[5]Division of Immunology and Allergy, University Hospital Lausanne, Lausanne, Switzerland

[6]Department of Infectious Diseases, Berne University Hospital and University of Berne, Berne, Switzerland

[7]Division of Infectious Diseases and Hospital Epidemiology, University Hospital Basel, Basel, Switzerland

[8]Division of Infectious Diseases, University Hospital Lausanne, Lausanne, Switzerland

[9]Division of Infectious Diseases, Regional Hospital Lugano, Lugano, Switzerland

[10]Division of Infectious Diseases, Cantonal Hospital St. Gallen, St. Gallen, Switzerland

[11]Institute of Social and Preventive Medicine, University of Zurich, Zurich, Switzerland

Corresponding author: Huldrych F. Günthard, University Hospital Zurich, Division of Infectious Diseases and Hospital Epidemiology; Rämistrasse 100, 8092 Zurich, Switzerland, e-mail: huldrych.guenthard@usz.ch, phone number: +41 44 255 34 50, fax number: +41 44 255 32 91

Abstract

Background

Transmitted HIV-1 drug-resistance mutations(TDR) are transmitted from treatment-failing or treatment-naïve patients. Although prevalence of drug-resistance in treatment-failing patients has declined in developed countries, TDR prevalence has not. Mechanisms causing this paradox are poorly explored.

Methods

We included recently-infected, treatment-naïve patients with genotypic-resistance-tests performed ≤1year post-infection and <2013. Potential risk factors for TDR were analyzed using logistic regression. Association of TDR prevalences with population viral load(PVL) from treatment-patients during 1997-2011 was estimated with Poisson regression for all TDR and individually for most frequent resistance-mutations against each drug class(M184V/L90M/K103N).

Results

We included 2421 recently-infected, treatment-naïve patients and 5399 treatment-failing patients. TDR prevalence fluctuated considerably over time. Two opposing developments could explain these fluctuations: generally continuous increases in TDR(Odds Ratio[OR]=1.13,p=0.010), punctuated by sharp decreases when new drug-classes were introduced. Overall, TDR prevalence increased with decreasing PVL(Rate Ratio[RR]=0.91/1000Log_{10}-PVL,p=0.033). Additionally, we observed that the transmitted high-fitness-cost mutation M184V was positively associated with PVL of treatment-failing patients carrying M184V(RR=1.50/100Log_{10}-PVL,p<0.001). Such association was absent and negative for K103N(RR-K103N=1.00/100Log_{10}-PVL,p=0.99) and L90M(RR-L90M=0.75/100Log_{10}-PVL,p=0.022), respectively.

Conclusions

Transmission of antiretroviral drug-resistance is temporarily reduced by the introduction of new drug classes and driven by treatment-failing and treatment-naïve patients. These findings suggest a continuous need for new drugs, early detection/treatment of HIV-1-infection.

Introduction

Transmission of HIV-1 infection depends strongly on individual levels of plasma viremia [1]. When HIV-1-infected patients receive suboptimal treatment or incomplete adherence to anti-retroviral therapy (ART), drug-resistant viruses emerge and continue replicating. Therefore, the general assumption is that drug-resistant viruses are mainly transmitted from treated patients with high levels of HIV viremia due to failed treatment [2]. Modern ART reduces the viremia levels and transmissibility of HIV-1 more effectively than earlier ART [3], suggesting less emergence [4] and transmission of HIV-1 drug-resistance over time.

In recent years, the incidence and prevalence of acquired drug-resistance mutations (ADRs) in treated patients has indeed declined due to effective ART in various developed countries [5,6]. However, prevalence of transmitted drug-resistance mutations (TDR) has often remained stable [7-9]. TDR may cause early virological failure when patients start their first-line therapy [10]. Certain TDR can persist for years in the absence of drug pressure after seroconversion [11] and have long-term potential to jeopardize the effectiveness of ART; other TDR may disappear rapidly and become undetectable via population sequencing [11,12]. Recently, transmission of minority variants harboring drug-resistance has been demonstrated [13]. Difficulties in detecting TDR upon ART-initiation might therefore compromise the treatment success achieved thus far.

In the current study we aimed at analysing the risk factors of TDR, and resolving the discrepant patterns of TDR and ADR prevalence over time. The unique SHCS dataset, which is representative for ≥15 years, allows us to determine the impact of temporarily changing factors such as numbers of available drug classes. We adapted population viral load (PVL) as a tool to assess the spread of drug-resistance and the transmission potential of the treatment-experienced population. We focused specifically on TDR during recent infections to avoid potential bias caused by different TDR persistence times.

Methods

Study population

The SHCS, enrolling patients since 1988, is a prospective, nationwide, clinic-based study including a biobank. The SHCS is representative for the HIV epidemiology in Switzerland; it includes at least 53% of all HIV cases ever diagnosed in Switzerland, 72% of all patients receiving ART, and 69% of the nationwide registered AIDS cases [14]. Additionally, we enrolled patients from the Zurich Primary HIV-infection study (ZPHI:www.clinicaltrials.gov; ID=NCT00537966) which focuses on identifying and treating patients during early infection [15]. Ethical approval from all participating institutions and written informed consent from all patients was obtained [14-16].

To identify the TDR prevalence, we included recently-infected, treatment-naïve patients (definition:see below) with a genotypic resistance test (GRT) performed before 1.1.2013. The first GRT from each recently-infected, treatment-naïve individual was considered. All sequences before 1996 were grouped as ≤1995 because of small sample sizes. For the association analysis, in which we tested whether TDR prevalence is associated with the PVL from ART-failing patients, we included ART-failing patients from 1997-2011 due to the representative availability of VL testing since 1997.

GRTs stem from routine-clinical testing performed by four laboratories in Switzerland authorized by the Federal Office of Public Health. All laboratories perform population-based sequencing of the full protease gene and at least codons 28–225 of the reverse transcriptase gene using commercial assays (Viroseq Vs.1 PE Biosystems;Virsoseq Vs. 2, Abbott AG; vircoTYPE HIV-1 Assay, Virco Lab) and in-house methods [17] and participate in the yearly quality control evaluation by the Agence Nationale de la Recherche du SIDA (ANRS) since 2002. All sequences are entered into the SHCS drug-resistance database using SmartGene's Integrated Dababase Network System (SmartGene, Zug, Switzerland, IDNS

version 3.6.3) [18]. Additionally, we performed systematically retrospective sequencing for blood samples that were stored in the biobank before routine genotyping was introduced (over 11000 sequences were retrospectively generated). Subtyping was performed on the protease and the reverse transcriptase sequence using REGA 2 (http://jose.med.kuleuven.be/genotypetool/html/subtypinghiv.html). If this method returned inconclusive results, the analysis was repeated with the Star analyzer (http://www.vgb.ucl.ac.uk/starn.shtml) [19].

TDR were identified using the WHO list for surveillance of transmitted HIV drug-resistance [20].

Definition of recent infection

To account for potential reversion of TDR in the absence of drug pressure [11,21-25], we restricted our study population to treatment-naïve patients having been diagnosed ≤1 year after infection. Specifically, we determined recent infection with one of the following methods:

(1) Documented acute HIV-1 infection as previously described [15].

(2) Documented seroconversion (<1 year between the last negative and first positive HIV tests).

(3) For those lacking the data mentioned above, the ambiguity score [26] was used. It is a measure of the viral nucleotide diversity from bulk sequencing which estimates the infection duration. Sequences with ≤0.5% ambiguous nucleotides were considered to be GRTs from recently-infected patients [26]. However, as diversity may be low in long-term HIV-infections, patients with a score ≤0.5% and a CD4 count <200 were excluded to reduce false positives. For validation of this method, see Supplementary Material.

The viral burden of treatment-failing patients

PVL was used to describe the viral burden of ART-failing patients for the coming year on a population level. We summed up the $\log_{10}$ transformed VLs from all ART-failing patients of a

given year. For further analyses, where we studied the transmission pattern of a specific TDR, the total of $\log_{10}$ transformed VLs from ART-failing patients carrying the corresponding mutation was used. Only VLs corresponding to a GRT were included for these analyses because genotyping was needed to determine drug-resistance mutations.

To acquire all potential treatment failures, we defined treatment failure as having a VL ≥400 copies/mL after 180 days of continuous ART. VL measurement was not fully integrated into the clinical routine before 1997, so we included VLs from treatment-failing patients during 1997-2011. Each person contributed to each year once. If a patient had ≥2 VLs measured within the same year, we calculated the mean for that year.

Statistical methods

Potential risk factors for acquiring any TDR were analyzed using logistic regression. Variables investigated were ethnicity (Caucasian, Black, others), gender (male, female), transmission group (men having sex with men [MSM], heterosexual transmission [HSX], injecting drug users [IDU], others), HIV-1 subtype (B, non-B), and the calendar year of sampling (fitted as a continuous variable). Additionally, since we suspected that less optimal regimens resulting from fewer choices of available drugs might have influenced TDR transmission, we included the number of available drug classes as an ordered categorical variable (the p-value was obtained from the test for trend). In Switzerland, HIV-1 treatment occurred in five eras, each separated by the introduction of a new drug class: Mono-class therapy with nucleoside analogue reverse transcriptase inhibitors (NRTI) was used before 1996 (1 drug class:≤1996). After the introduction of unboosted protease inhibitors (PI) in 1996, patients could obtain dual-class regimens (2 classes:1997-1998). Subsequently, non-nucleoside analogue reverse transcriptase inhibitors (NNRTI) were introduced in 1998 (3 classes:1999-2000), followed by boosted PI (PI/r) in 2000 (4 classes:2001-2008), and integrase inhibitor (InSTI) in 2008 (5 classes:2009-2012). In the model we included binary response indicating detection of any TDR from each patient as an outcome. We analyzed

variables independently and included those associated significantly with the outcome into the multivariable model (HIV subtype and transmission group). We also chose variables a priori regardless of univariable significance due to likely biological impacts (sex, year, and number of available drug classes). For TDR to individual drug classes, we included the same co-variables in the multivariable models for reasons of consistency to avoid obtaining a different set of variables for each drug class. We found no collinearity and interactions between any included variable. Missing data were list-wise deleted. We calculated odds of TDR detection from our fitted multivariable model by retaining all co-variables except for year and number of available drug classes at baseline, and transformed the predicted odds to annual prevalences.

In the association analysis we applied Poisson regression to assess the association of TDR transmission with treatment-failing patients as potential transmitters. We considered annual rates of GRTs detecting TDR from recently-infected, treatment-naïve patients as outcome and PVL of all treatment-failing patients from the previous year as explanatory variable. We further studied the association for the most prevalent drug-resistance mutation for each major drug class in the SHCS: M184V, L90M, and K103N for NRTI, PI, and NNRTI, respectively. In this individual-mutation analysis, we fitted the model with the annual prevalence of each of these three transmitted mutations as outcome and the PVL of ART-failing patients carrying the corresponding mutation from the previous year as explanatory variable. We performed sensitivity analyses including PVL from the same year of GRTs performed or from two years before (see Supplementary Material).

We expressed our results with 95% CI and two-sided p-values, with $p<0.05$ being statistically significant. Data analyses were performed with Stata 13.0 SE (StataCorp, Texas, USA).

Subgroup analysis

Considering that transmission to some SHCS patients may have occurred abroad and that the TDR prevalences of those patients would be less relevant to treatment-experienced

patients in Switzerland, we repeated the association analyses with only those patients found in Swiss transmission clusters, defined phylogenetically [27]. To summarize, HIV-1 subtype B *pol* sequences from 8271 SHCS patients were pooled with foreign *pol* sequences from the Los Alamos Sequence database (n=36230). Clusters were defined as clades containing ≥10 sequences and consisting of ≥80% sequences from the SHCS.

Results

Fraction of positive GRTs in the SHCS

Figure 1 summarizes the fraction of TDR and ADR from all 20120 GRTs sampled before 1.1.2013 regardless of the infection duration stratified by treatment status (naïve/experienced). Specifically, 10504 GRTs were from 7920 treatment-naïve and 9616 GRTs from 4816 treatment-experienced individuals.

ADR reached a peak at 85% in 1998 and dropped continuously since then to a plateau at ~38% in 2009. This strong decrease of fraction of positive GRTs for ADR (linear regression: -2.8% /year [-3.4%,-2.2%]; p<0.001) was not followed by a parallel decrease but rather a slight increase of fraction of positive GRTs for TDR (0.3% /year [0.2%,0.5%]; p<0.001). To further dissect this discrepancy and to avoid possible bias introduced by different persistence times of TDRs, we focused on studying treatment-naïve patients with GRTs performed within recent infection.

Study population including recently-infected, treatment-naïve and treatment-failing patients

We identified 2421 (31%) recently-infected patients from 7920 treatment-naïve patients in the SHCS with ≥1 GRT performed between June 26, 1992 and Dec.18, 2012. Additionally, we included 5399 patients having failed ≥ 1 regimen within years 1997-2011, presenting 18097

yearly-unique VL measurements. For detailed patient selection, see Figure 2. For representativeness of study population see Supplementary Figure S1.

TDR prevalences over time in recently-infected treatment-naïve patients and associated risk factors

TDR prevalences fluctuated substantially over time with the median (range) as follows: 9.1% (2.2%, 15.6%) to any drug; 5.8% (2.2%, 14.3%) to NRTI; 2.5% (0, 4.8%) to PI; 1.4% (0, 5.1%) to NNRTI (Figure 3, black dots).

We observed two opposing developments in the multivariable logistic model that could explain the complex fluctuations of TDR prevalences (Table 1). On the one hand, overall TDR prevalence dropped after introduction of new drug classes. In particular, prevalences of TDRs significantly dropped after PI/r and InSTI became available. On the other hand, we found a linear increase of TDR prevalences when the number of available drug classes remained constant (Figure 3). The combination of these two opposing developments resulted in TDR prevalences, which increased in the absence of new drugs but decreased sharply upon introduction of new drug classes. TDR prevalences predicted from this model were shown in Figure 3 (blue lines).

Additionally, prevalences of TDR to individual drug classes showed similar but not significant patterns as mentioned above (Supplementary Table S1.1-S1.3).

Association of drug-resistance transmission with the viral burden of treatment-failing patients

We further investigated whether drug-resistance transmission was associated with treatment-failing patients. We fitted annual prevalences of any TDR (outcome) and PVL of treatment-failing patients from the previous year (explanatory variable) with a Poisson regression model. The rate ratio (RR) was 0.91/1000 PVL (0.83, 0.99; p=0.033), indicating a 9%

increase of TDR prevalence for a decrease of PVL of ART-failing patients from the previous year by 1000 (Figure 4A,E). PVL itself decreased over time (linear regression: -318/year [-438,-197]; p<0.001). When we considered patients identified in Swiss transmission clusters, we found no discernible evidence for an association between TDR and PVL (RR=0.76/1000 PVL [0.43,1.34]; p=0.34).

Taken together, our results suggested no or a negative association between TDR prevalences and PVL of ART-failing patients from the previous year.

Transmission of the class specific drug-resistance mutations M184V, L90M, K103N

The above analysis pooled all TDRs and potentially neglected the differential behavior of individual mutations. We therefore performed individual-mutation analysis for the most prevalent drug-resistance mutation for each drug class.

Transmitted M184V increased 1.5 fold for an increase of PVL from ART-failing patients carrying M184V from the previous year by 100 (RR=1.50/100 PVL [1.20,1.86]; p<0.001; Figure 4B,F). This association increased to ~6 fold when only TDRs from Swiss transmission clusters were considered (RR=5.68/100 PVL [1.21,26.7]; p=0.028). On the contrary, we observed a negative association between the transmitted L90M and PVL from ART-failing patients carrying L90M from the previous year (RR=0.75/100 PVL [0.58,0.96]; p=0.022; Figure 4C,G); the association became stronger when TDRs from Swiss transmission clusters were considered (RR=0.07/100 PVL [0.01,0.46]; p=0.006). For K103N no association was detected (RR=1.00/100 PVL [0.73,1.37]; p=0.99; Figure 4D,H) and RR became negative when including only patients from Swiss transmission clusters but without reaching significance (RR=0.02/100 PVL [0.0002,1.55]; p=0.078).

Sensitivity analyses using PVL from different years, and validating the ambiguity score for identifying recent infections showed that our results were robust (Supplementary Table S2, S3.1, S3.2). For a summary of sample size and method used in each analysis see Supplementary Table 4.

Discussion

In this study we investigated the paradox between the decrease in ADR prevalence [5,6,28] and a nearly stable prevalence of TDR [7,8,29-31]. If TDR indeed primarily originate from ART-failing patients with ADR, this discrepancy is counterintuitive. We therefore tested whether transmission of drug-resistant viruses was dependent on ART-failing patients in the SHCS, which is representative for Switzerland, over a 15-year time period. A large, clearly defined recently-infected, treatment-naïve population was used to calculate TDR prevalences.

Our results indicate that drug-resistance transmission is not predominantly driven by treatment-failing patients, but rather by a complex mixture of both ART-failing and ART-naïve patients. Despite PVL of treatment-failing patients decreased continuously, TDR prevalences increased over time. When specific TDRs were studied individually, distinct transmission patterns emerged. The prevalence of transmitted M184V correlated positively with PVL from ART-failing patients carrying M184V from the previous year. This association became stronger for patients included in Swiss transmission clusters. This suggests that the treatment-failing population is the major transmission source for M184V. In contrast, no positive association was found for L90M or K103N. We detected a negative association between prevalences of transmitted L90M and PVL from ART-failing patients carrying L90M from the previous year. This implies that major transmission reservoirs for these mutations are treatment-naïve rather than treatment-failing patients.

How can we explain such divergent transmission patterns between specific drug-resistance mutations? It is most likely due to the differential fitness costs, which represent the reduced ability of a virus harboring a drug-resistance mutation to replicate in the absence of the drug to which the mutation confers resistance. Generally, drug-resistant viruses will be replaced gradually by fitter viruses when drug pressure is not present, and the rate of the replacement depends on the degree of the fitness cost [32]. M184V disappears at a fast rate after

transmission [11] without drug pressure due to its high fitness cost [33]. Therefore, M184V was rarely found in a drug-naïve population and its transmission depends on treatment-failing patients. In contrast, low-fitness-cost mutations L90M and K103N [23,34,35] persist longer in the absence of drug pressure [23], and may therefore persist within the ART-naïve population, which thus becomes an important source for transmission of these mutations. This interpretation is further supported by the fact that occurrence of L90M among recently-infected, treatment-naïve patients has increased years after the PVL from ART-failing patients carrying L90M started to decrease (Figure 4), resulting in the negative association from the Poisson regression. A similar but weaker phenomenon was observed for K103N. Various combinatorial ART-regimens might contribute to differences between transmission patterns of L90M and K103N. Drugs selecting for L90M, mainly saquinavir and nelfinavir, have been almost unused in Switzerland for many years, indicating circulation of transmitted L90M within the treatment-naïve population. On the other hand, drugs selecting for K103N, such as efavirenz and nevirapine, are still in heavy use, implying that transmission of K103N is fueled both by treatment-failing and treatment-naïve patients.

Complemented by results from previous phylogenetic analyses [36-38], our study further illustrates that the treatment-naïve population is a major source for ongoing transmission of low-fitness-cost mutations. Early diagnosis and treatment of HIV-1-infected individuals is warranted to block the otherwise self-fueling mechanism of unrecognized TDR, which persist in this population due to low fitness costs.

In the SHCS TDR prevalences fluctuated considerably over time. We hypothesized that introductions of new drugs had an effect on these fluctuations, because new drugs improve control of viremia in treated patients. Indeed, after each introduction of a new drug class, a drop in TDR prevalences was observed: in 1997 after introduction of PI, 1999 after NNRTI, 2001 after PI/r, and 2009 after InSTI (Figure 3). Despite the universal and unlimited access to ART in Switzerland, TDR prevalences could not be reduced over an 18-year study period

(Figure 3A). Possibly, even more TDRs would have occurred without a constant influx of new therapy options. This highlights the importance of a drug pipeline that constantly delivers new medications.

There are several limitations to this study. Although our study was limited to a single country, we believe that our findings are generalizable to settings with similar HIV epidemics and treatment policies (for generalizability see Supplementary Material). In the correlation analyses we used measures of treatment-failing patients from the previous year because we assumed that treatment-failing patients could transmit drug-resistance approximately within one year before salvage treatment is fully active. Sensitivity analyses using PVL from the same year or two years before revealed similar results to the original model (Supplementary Table S2). Furthermore, the lack of positive associations from individual-mutation analyses of L90M/K103N does not causally prove that treatment-naïve individuals are the main source for the transmission. Though unlikely due to the well-studied transmission dynamics within the SHCS [27], we cannot exclude that patients carrying the transmitted L90M/K103N in our study population might all have been infected abroad and thus the ART-failing PVL as measured in the SHCS would not be relevant. However, the subgroup analysis including only patients from Swiss transmission clusters confirmed the same finding.

Conclusions

We demonstrated that transmission of antiretroviral drug-resistance is temporarily reduced by the introduction of new drug classes and driven both by treatment-failing and treatment-naïve patients. These findings suggest a continuous need for new drugs, early detection and early treatment of HIV-1 infection to successfully control the spread of TDR in the long term.

Acknowledgments

We thank the patients who participate in the SHCS; the physicians and study nurses for excellent patient care; the resistance laboratories for high-quality genotypic drug-resistance testing; SmartGene, Zug, Switzerland, for technical support; Brigitte Remy, RN, Martin Rickenbach, MD, Franziska Schöni-Affolter, MD, and Yannick Vallet, MSc from the SHCS Data Center in Lausanne for data management; and Danièle Perraudin and Mirjam Minichiello for administrative assistance.

The members of the SHCS are:
Aubert V, Battegay M, Bernasconi E, Böni J, Bucher HC, Burton-Jeangros C, Calmy A, Cavassini M, Dollenmaier G, Egger M, Elzi L, Fehr J, Fellay J, Furrer H (Chairman of the Clinical and Laboratory Committee), Fux CA, Gorgievski M, Günthard H (President of the SHCS), Haerry D (deputy of "Positive Council"), Hasse B, Hirsch HH, Hoffmann M, Hösli I, Kahlert C, Kaiser L, Keiser O, Klimkait T, Kouyos R, Kovari H, Ledergerber B, Martinetti G, Martinez de Tejada B, Metzner K, Müller N, Nadal D, Nicca D, Pantaleo G, Rauch A (Chairman of the Scientific Board), Regenass S, Rickenbach M (Head of Data Center), Rudin C (Chairman of the Mother & Child Substudy), Schöni-Affolter F, Schmid P, Schüpbach J, Speck R, Tarr P, Telenti A, Trkola A, Vernazza P, Weber R, Yerly S.

Footnotes

This work was presented in part at the International Workshop on HIV & Hepatitis Virus Drug Resistance and Curative Strategies, Toronto, 2013, June 4-8, 2013, Abstract # A21.

Contact information of corresponding author

Huldrych F. Günthard, University Hospital Zurich, Division of Infectious Diseases and Hospital Epidemiology; Rämistrasse 100, 8092 Zurich, Switzerland

e-mail: huldrych.guenthard@usz.ch

phone number: +41 44 255 34 50, fax number: +41 44 255 32 91

Funding statement

This study has been financed in the framework of the Swiss HIV Cohort Study, supported by the Swiss National Science Foundation (SNF grant #33CS30-134277) and the SHCS projects #470, 528, 569, 683, the SHCS Research Foundation, the Swiss National Science Foundation (grant # 324730-130865 (to HFG), the European Community's Seventh Framework Program (grant FP7/ 2007–2013), under the Collaborative HIV and Anti-HIV Drug-resistance Network (CHAIN; grant 223131, to HFG), by the Yvonne-Jacob foundation,and by a further research grant of the Union Bank of Switzerland, in the name of an anonymous donor to HFG, an unrestricted research grant from Gilead, Switzerland to the SHCS research foundation, and by the University of Zurich's Clinical research Priority Program (CRPP) "Viral infectious diseases: Zurich Primary HIV Infection Study" (to HFG). R. Kouyos was supported by SNF # PZ00P3-142411. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Potential conflicts of interest

H.F.G. has been an adviser and/or consultant for the following companies: GlaxoSmithKline, Abbott, Gilead, Novartis, Boehringer Ingelheim, Roche, Tibotec, Pfizer and Bristol-Myers Squibb, and has received unrestricted research and educational grants from Roche, Abbott, Bristol-Myers Squibb, Gilead, Astra-Zeneca, GlaxoSmithKline, and Merck Sharp & Dohme (all money went to institution).

E.B. has been consultant for BMS, Gilead, ViiV Healthcare, Pfizer, MSD, and Janssen. He has received unrestricted research grants from Gilead, Abbott, Roche and MSD. He also received travel grants from BMS, Boehringer Ingeldheim, Gilead, MSD, and Janssen.

S.Y. has been consultant for BMS, has received unrestricted research and educational grants from Roche, ViiV and Gilead.

T.K. served as advisor for Bristol-Myers Squibb and Pfizer and has re- ceived travel grants from Abbott and Pfizer.

L.H. has been consultant for Roche and Nycomed (now owned by Takeda Pharmaceutica).

References

1. Quinn TC, Wawer MJ, Sewankambo N, et al. Viral load and heterosexual transmission of human immunodeficiency virus type 1. Rakai Project Study Group. N. Engl. J. Med. **2000**; 342:921–929.

2. Hirsch MS, Conway B, D'Aquila RT, et al. Antiretroviral drug resistance testing in adults with HIV infection: implications for clinical management. International AIDS Society--USA Panel. 1998: 1984–1991.

3. Porco TC, Martin JN, Page-Shafer KA, et al. Decline in HIV infectivity following the introduction of highly active antiretroviral therapy. AIDS **2004**; 18:81–88.

4. Wyl von V, Yerly S, Böni J, et al. Incidence of HIV-1 drug resistance among antiretroviral treatment-naive individuals starting modern therapy combinations. Clin Infect Dis **2012**; 54:131–140.

5. Wyl von V, Yerly S, Böni J, et al. Long□Term Trends of HIV Type 1 Drug Resistance Prevalence among Antiretroviral Treatment–Experienced Patients in Switzerland. Clin Infect Dis **2009**; 48:979–987.

6. Andrea De Luca, Dunn D, Zazzi M, et al. Declining Prevalence of HIV-1 Drug Resistance in Antiretroviral Treatment-exposed Individuals in Western Europe. J Infect Dis **2013**; 207:1216–1220.

7. UK Collaborative Group on HIV Drug Resistance. Time trends in drug resistant HIV-1 infections in the United Kingdom up to 2009: multicentre observational study. BMJ **2012**; 345:e5253.

8. Yerly S, Wyl von V, Ledergerber B, et al. Transmission of HIV-1 drug resistance in Switzerland: a 10-year molecular epidemiology survey. AIDS **2007**; 21:2223–2229.

9. Frentz D, Boucher CAB, van de Vijver DAMC. Temporal changes in the epidemiology of transmission of drug-resistant HIV-1 across the world. AIDS Rev **2012**; 14:17–27. Available at: http://aidsreviews.com/audita.asp?d=2012_14_1_017-027.pdf&t=D&w=/resumen.asp&q=id=1163&indice=2012141&u=unp.

10. Wittkop L, Günthard HF, de Wolf F, et al. Effect of transmitted drug resistance on virological and immunological response to initial combination antiretroviral therapy for HIV (EuroCoord-CHAIN joint project): a European multicohort study. The Lancet Infectious Diseases **2011**; 11:363–371.

11. Jain V, Sucupira MC, Bacchetti P, et al. Differential Persistence of Transmitted HIV-1 Drug Resistance Mutation Classes. J Infect Dis **2011**; 203:1174–1181.

12. Li JZ, Paredes R, Ribaudo HJ, et al. Low-frequency HIV-1 drug resistance mutations and risk of NNRTI-based antiretroviral treatment failure: a systematic review and pooled analysis. JAMA **2011**; 305:1327–1335.

13. Metzner KJ, Scherrer AU, Preiswerk B, et al. Origin of Minority Drug-Resistant HIV-1 Variants in Primary HIV-1 Infection. J Infect Dis **2013**; 208:1102–1112.

14. Swiss HIV Cohort Study, Schoeni-Affolter F, Ledergerber B, et al. Cohort profile: the Swiss HIV Cohort study. Int J Epidemiol **2010**; 39:1179–1189.

15. Rieder P, Joos B, Scherrer AU, et al. Characterization of Human Immunodeficiency Virus Type 1 (HIV-1) Diversity and Tropism in 145 Patients With Primary HIV-1 Infection. Clin Infect Dis **2011**;

16. Ledergerber B, Egger M, Opravil M, et al. Clinical progression and virological failure on highly active antiretroviral therapy in HIV-1 patients: a prospective cohort study. Swiss HIV Cohort Study. Lancet **1999**; 353:863–868.

17. Yerly S, Vora S, Rizzardi P, et al. Acute HIV infection: impact on the spread of HIV and transmission of drug resistance. AIDS **2001**; 15:2287–2292.

18. Wyl von V, Yerly S, Böni J, et al. Emergence of HIV-1 drug resistance in previously untreated patients initiating combination antiretroviral treatment: a comparison of different regimen types. Arch. Intern. Med. **2007**; 167:1782–1790.

19. Myers RE, Gale CV, Harrison A, Takeuchi Y, Kellam P. A statistical model for HIV-1 sequence classification using the subtype analyser (STAR). Bioinformatics **2005**; 21:3535–3540.

20. Bennett DE, Camacho RJ, Otelea D, et al. Drug resistance mutations for surveillance of transmitted HIV-1 drug-resistance: 2009 update. PLoS ONE. 2009; 4:e4724.

21. Brenner BG, Routy JP, Petrella M, et al. Persistence and Fitness of Multidrug-Resistant Human Immunodeficiency Virus Type 1 Acquired in Primary Infection. Journal of Virology **2002**; 76:1753–1761.

22. Pao D, Andrady U, Clarke J, et al. Long-term persistence of primary genotypic resistance after HIV-1 seroconversion. J Acquir Immune Defic Syndr Hum Retrovirol **2004**; 37:1570–1573.

23. Little SJ, Frost SDW, Wong JK, et al. Persistence of Transmitted Drug Resistance among Subjects with Primary Human Immunodeficiency Virus Infection. Journal of Virology **2008**; 82:5510–5518.

24. Jayaraman GC, Archibald CP, Kim J, et al. A Population-Based Approach to Determine the Prevalence of Transmitted Drug-Resistant HIV Among Recent Versus Established HIV Infections: Results From the Canadian HIV Strain and Drug Resistance Surveillance Program. J Acquir Immune Defic Syndr Hum Retrovirol **2006**; 42:86–90.

25. Devereux HL, Youle M, Johnson MA, Loveday C. Rapid decline in detectability of HIV-1 drug resistance mutations after stopping therapy. AIDS **1999**; 13:F123–7.

26. Kouyos RD, Wyl von V, Yerly S, et al. Ambiguous Nucleotide Calls From Population-based Sequencing of HIV-1 are a Marker for Viral Diversity and the Age of Infection. Clin Infect Dis **2011**; 52:532–539.

27. Kouyos RD, Wyl von V, Yerly S, et al. Molecular epidemiology reveals long-term changes in HIV type 1 subtype B transmission in Switzerland. J Infect Dis **2010**; 201:1488–1497.

28. Dunn D, Geretti AM, Green H, et al. Population trends in the prevalence and patterns of protease resistance related to exposure to unboosted and boosted protease inhibitors. Antiviral Therapy **2008**; 13:771–777.

29. Castor D, Low A, Evering T, et al. Transmitted drug resistance and phylogenetic relationships among acute and early HIV-1-infected individuals in New York City. J Acquir Immune Defic Syndr Hum Retrovirol **2012**; 61:1–8.

30. Manasa J, Katzenstein D, Cassol S, Newell M-L, de Oliveira T, Southern Africa Treatment And Resistance Network (SATuRN). Primary drug resistance in South Africa: data from 10 years of surveys. AIDS Res Hum Retroviruses **2012**; 28:558–565.

31. Bartmeyer B, Kuecherer C, Houareau C, et al. Prevalence of Transmitted Drug Resistance and Impact of Transmitted Resistance on Treatment Success in the German HIV-1 Seroconverter Cohort. PLoS ONE **2010**; 5:e12718.

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

33. Paredes R, Sagar M, Marconi VC, et al. In Vivo Fitness Cost of the M184V Mutation in Multidrug-Resistant Human Immunodeficiency Virus Type 1 in the Absence of Lamivudine. Journal of Virology **2009**; 83:2038–2043.

34. Cong M-E, Heneine W, García-Lerma JG. The fitness cost of mutations associated with human immunodeficiency virus type 1 drug resistance is modulated by mutational interactions. Journal of Virology **2007**; 81:3037–3041.

35. Armstrong KL, Lee TH, Essex M. Replicative Fitness Costs of Nonnucleoside Reverse Transcriptase Inhibitor Drug Resistance Mutations on HIV Subtype C. Antimicrobial Agents and Chemotherapy **2011**; 55:2146–2153.

36. Yerly S, Junier T, Gayet-Ageron A, et al. The impact of transmission clusters on primary drug resistance in newly diagnosed HIV-1 infection. AIDS **2009**; 23:1415–1423.

37. Ambrosioni J, Junier T, Delhumeau C, et al. Impact of highly active antiretroviral therapy on the molecular epidemiology of newly diagnosed HIV infections. AIDS **2012**; 26:2079–2086.

38. Drescher SM, Wyl von V, Yang WLL, et al. Treatment-Naive Individuals Are the Major Source of Transmitted HIV-1 Drug Resistance in Men Who Have Sex With Men in the Swiss HIV Cohort Study. Clin Infect Dis **2014**; 58:285–294.

Table 1: Univariable and multivariable analysis for the overall TDR prevalences[a]

	No. with resistance / Total No. in subgroup (%)[b,c]	OR (95% CI) in univariable analysis	p-value	OR (95% CI) in multivariable analysis	p-value
Age	35 (28, 42)[c]	1.00 (0.98 - 1.01)	0.62		
Ethnicity			0.33		
Caucasian	182/1985 (9.2)	1.00 (Ref.)			
Black	16/222 (7.2)	0.77 (0.45 - 1.31)			
Others[d]	9/148 (6.1)	0.64 (0.32 - 1.28)			
HIV Subtype			<0.01		0.03
B	167/1683 (9.9)	1.00 (Ref.)		1.00 (Ref.)	
Non-B	40/672 (6.0)	0.57 (0.40 - 0.82)		0.65 (0.43 - 0.98)	
Sex			0.07		0.10
Male	173/1853 (9.3)	1.00 (Ref.)		1.00 (Ref.)	
Female	34/502 (6.8)	0.71 (0.48 - 1.03)		0.96 (0.60 - 1.55)	
Transmission Group[e]			0.03		0.62
MSM	129/1248 (10.3)	1.00 (Ref.)		1.00 (Ref.)	
HSX	52/770 (6.8)	0.63 (0.45 - 0.88)		0.83 (0.52 - 1.30)	
IDU	22/263 (8.4)	0.79 (0.49 - 1.27)		0.86 (0.51 - 1.45)	
Others	4/74 (5.4)	0.50 (0.18 - 1.38)		0.57 (0.20 - 1.60)	
No. of available drug classes[f]			0.77		0.06[g]
1 (NRTI)	10/125 (8.0)	0.97 (0.49 - 1.91)		2.99 (0.99 - 9.02)	
2 (NRTI,PI)	20/220 (9.1)	1.12 (0.68 - 1.84)		2.85 (1.19 - 6.83)	
3 (NRTI,PI,NNRTI)	25/235 (10.6)	1.33 (0.84 - 2.11)		2.75 (1.36 - 5.55)	
4 (NRTI,PI,NNRTI,PI/r)	103/1252 (8.2)	1.00 (Ref.)		1.00 (Ref.)	
5 (NRTI,PI,NNRTI,PI/r,InSTI)	49/523 (9.4)	1.15 (0.81 - 1.65)		0.61 (0.34 - 1.07)	
Year	2005 (2001, 2008)[c]	1.02 (0.98 – 1.05)	0.32	1.13 (1.03 – 1.23)[h]	0.01

a. We used logistic regression to model the odds of being detected as carrying TDR. The dependent variable was included as a binary response indicating whether any TDR was detected. All co-variables were categorical except for age and year that were continuous variables. In the multivariable model, we included significant variables from a univariable model: HIV subtype and transmission group. Variables chosen a priori to be included regardless of univariable significance were sex, number of available drug classes, and calendar year. Missing data were list-wise deleted, resulting that 66/2421=2.7% of patients were deleted due to missing subtype.
b. Number of patients with any drug resistance from the recently-infected, treatment-naïve patients with a clearly defined subtype (n = 2355).
c. For age and year, median (IQR) was shown
d. Others includes Asian, Hispanic, others, and unknown
e. MSM: men having sex with men, HSX: heterosexual, IDU: intravenous drug users, Others: others and unknown
f. NRTI: nucleotide reverse transcriptase inhibitor; PI: protease inhibitor; NNRTI: non-nucleotide reverse transcriptase inhibitor; PI/r: boosted protease inhibitor; InSTI: integrase inhibitor
g. p-value was obtained from the test for trend.
h. increment is per year

Figure legends

Figure 1: Fraction of positive GRTs detecting any drug-resistance mutation for acquired and transmitted drug-resistance in the SHCS

20120 GRTs were generated in total before 1.11.2013 in the SHCS. 10504 GRTs (blue triangles) were performed from 7920 patients when they were treatment-naïve (regardless of recent infection), and 9616 GRTs (red dots) from 4816 individuals when they were treatment-experienced. Fractions of positive GRTs detecting any drug-resistance mutation for both populations were shown. The annual numbers of included GRTs from treatment-experienced (first row, red) and from treatment-naïve (second row, blue) patients were listed below the graph. Linear regression with fraction as dependent variable and year as explanatory variable showed that the fraction of positive drug-resistance tests in treatment-experienced patients has declined substantially over time (-2.8% per year [-3.4%,-2.2%]; p<0.001), whereas the fraction of positive drug-resistance tests in treatment-naïve patients has not (0.3% per year [0.2%,0.5%]; p<0.001).

vertical bars = 95% CI

Figure 2: Patient selection profile

Numbers outside of the box indicate exclusions. (A) Selection profile for the recently-infected, treatment-naïve population. We selected patients enrolled in the SHCS before 1.1.2013 with GRTs performed when they were treatment-naïve (n=7920). From them we identified patients having GRTs performed during recent infection (≤1 year of infection) according to documented infection dates, seroconversions, or ambiguity score and CD4 count. These patients thus constitute our basic study population (n=2421). For further analyses such as for the uni- and multivariable analysis in Table 1 and for the association analyses in Figure 4A-4D, 66 and 252 patients were excluded due to additional criteria set for these analyses. 66 patients did not have a clearly defined subtype, and 252 patients were not sampled between 1998-2012 (for details see individual descriptions in Table 1 and Figure 4). (B) Selection

profile for the treatment-failing population. We chose PVL as an indicator for the viral burden from treatment-failing patients on a population level. PVL was defined as the sum of $\log_{10}$ transformed VLs from treatment-failing patients. We thus selected available VL measurements from SHCS patients when they were treatment-experienced. High VLs (≥ 400 copies/ml) measured after 180 days of and during a continuous therapy were included from these patients. Because VL has been fully integrated into clinical routine since 1997, values before 1997 were excluded. We calculated a yearly-unique VL from each patient (if ≥ 1 VL was available per patient within the same year, the mean was used) and used these values for association analysis in Figure 4E. For further association analyses as in Figure 4F-4H, where we studied the transmission pattern of a specific TDR, only VLs corresponding with a GRT were included because genotyping was needed to determine drug-resistance mutations. From VLs having corresponding GRTs we selected those from patients carrying M184V, L90M, or K103N for association analysis in Figure 4F, 4G, or 4H, respectively.

Figure 3: Observed and predicted TDR prevalences

2421 recently-infected, treatment-naïve patients with their first GRTs were included. For each year we calculated the percentage of GRTs detecting TDR (black dots) to (A) any drug, (B) NRTI, (C) PI, and (D) NNRTI. Additionally, we predicted TDR prevalence (blue dashed lines) by holding all co-variables except for year and number of available drug classes at baseline from the multivariable logistic regression model (Table 1) and transforming the odds obtained from the model. Co-variables included in the model were HIV-1 subtype and transmission group due to univariable significance and sex, number of available drug classes, and calendar year that were chosen a priori. Missing data were list-wise deleted. Total numbers of GRTs included for each year were listed at the bottom of Figure 3 (for observed data in black; for predicted data in blue). The reason for a smaller sample size (n=2355) for the predicted prevalences was that 66 patients were excluded from the multivariable model due to non-classified HIV-1 subtypes.

We found that the large fluctuations of the observed TDR prevalences (black dots) could be explained by two opposing developments: (1) a continuous increase with time when no new drug classes were introduced, and (2) a sharp decrease when a new drug class was introduced (orange vertical lines). This combined effect was described by the predicted TDR prevalence (blue dashed lines).

vertical bars = 95% CI

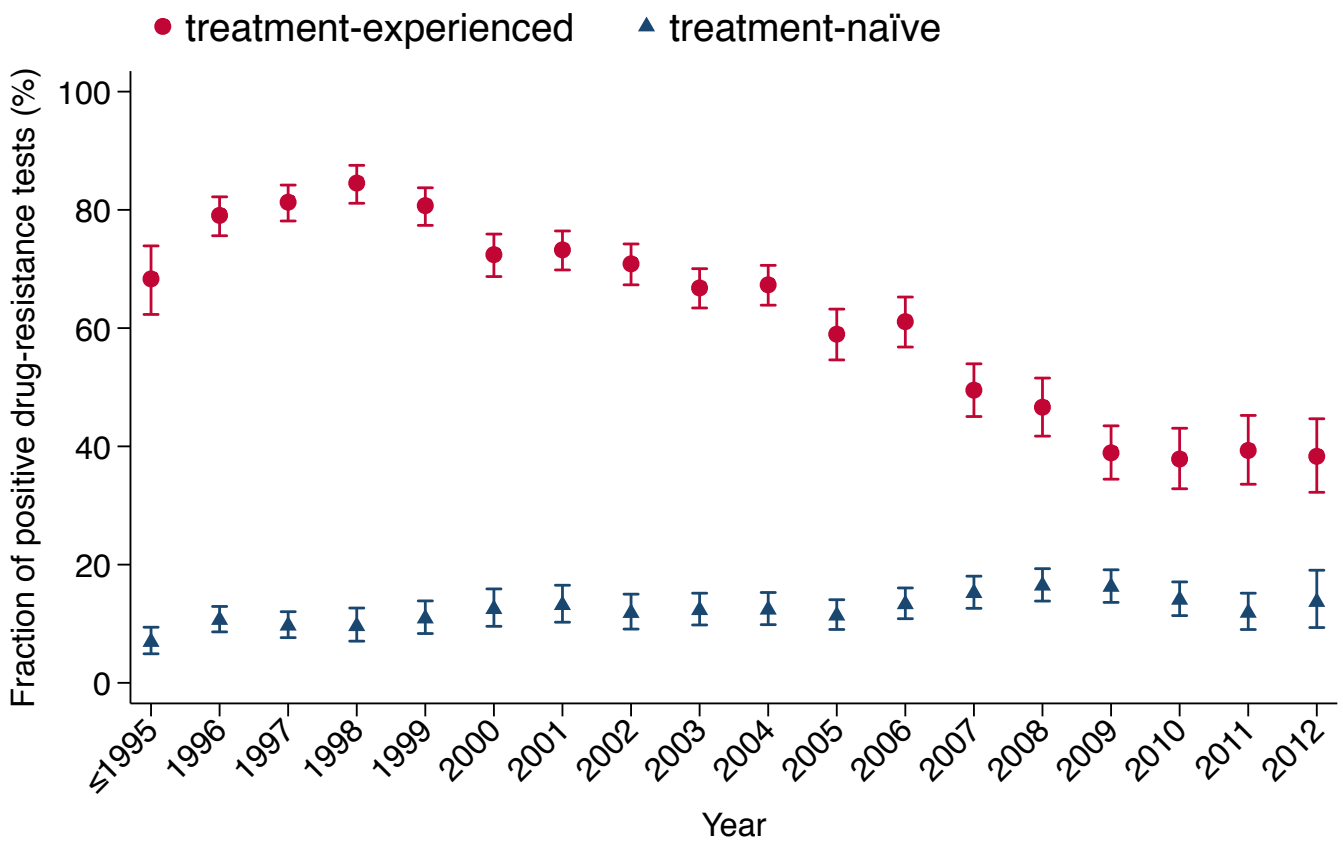
Figure 4: Association analysis for TDR prevalences with PVL from ART-failing patients from the previous year

Poisson regression was used to test the association between TDR and PVL from ART-failing patients from the previous year. 2169 patients with recently-infected, treatment-naïve GRTs during years 1998-2012 were included as the outcome to account for annual prevalences of (A) any TDR, (B) transmitted M184V, (C) transmitted L90M, and (D) transmitted K103N. Included as an explanatory variable was (E) PVL of all ART-failing patients, PVL of ART-failing patients carrying (F) M184V, (G) L90M, and (H) K103N, respectively, during years 1997-2011. Total numbers of GRTs performed from recently-infected, treatment-naïve patients for each year were listed in the first row of the table at the bottom of Figure 4. Annual numbers of yearly-unique VLs for all failing patients, noted as PVL (all failing patients), and PVL (failing patients with a specific mutation) were listed in the second to forth row.

We found that PVL of all treatment-failing patients has decreased over time (E; linear regression: -318 per year[-438,-197]; $p<0.001$). Annual prevalences for any TDR was negatively associated with PVL of treatment-failing patients from the previous year (A,E; RR=0.91 for every 1000 PVL-all increment [0.83,0.99]; p=0.033). Prevalence of transmitted M184V was positively associated with PVL from ART-failing patients carrying M184V from the previous year (B,F; RR=1.50 for every 100 PVL increment [1.20,1.86]; $p<0.001$). On the other hand, a negative association and no association was found for L90M (C,G; RR=0.75 for

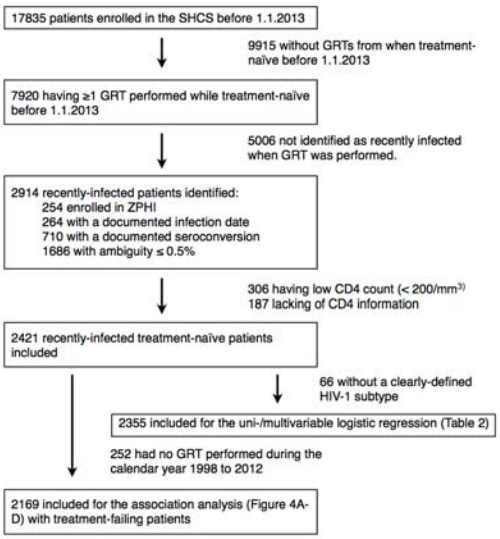
per 100 PVL increment [0.58,0.96]; p=0.022) and K103N (D,H; RR=1.00 for per 100 PVL increment [0.73,1.37]; p=0.99), respectively.

Figure 1



Year	≤ 95	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012
experienced n=9616	262	616	663	517	622	620	721	690	798	771	524	532	503	414	468	362	285	248
naïve n=10504	535	837	744	459	524	449	479	491	610	598	651	692	705	736	721	605	456	212

Figure 2(A) Selection profile of recently-infected, treatment-naïve patients



2(B) Selection profile of treatment-experienced patients for PVL

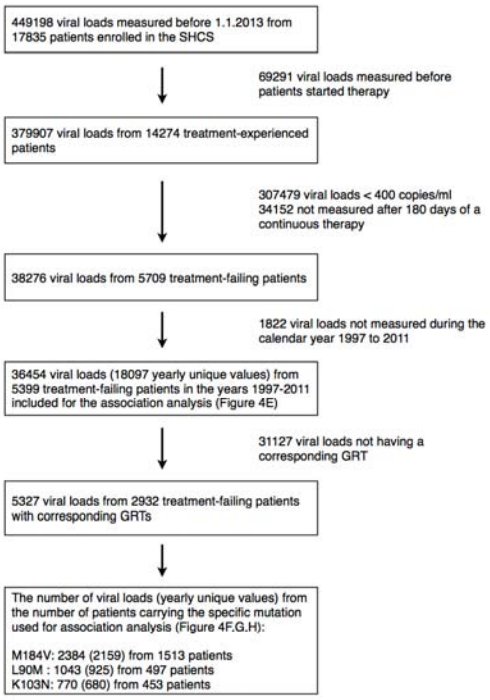
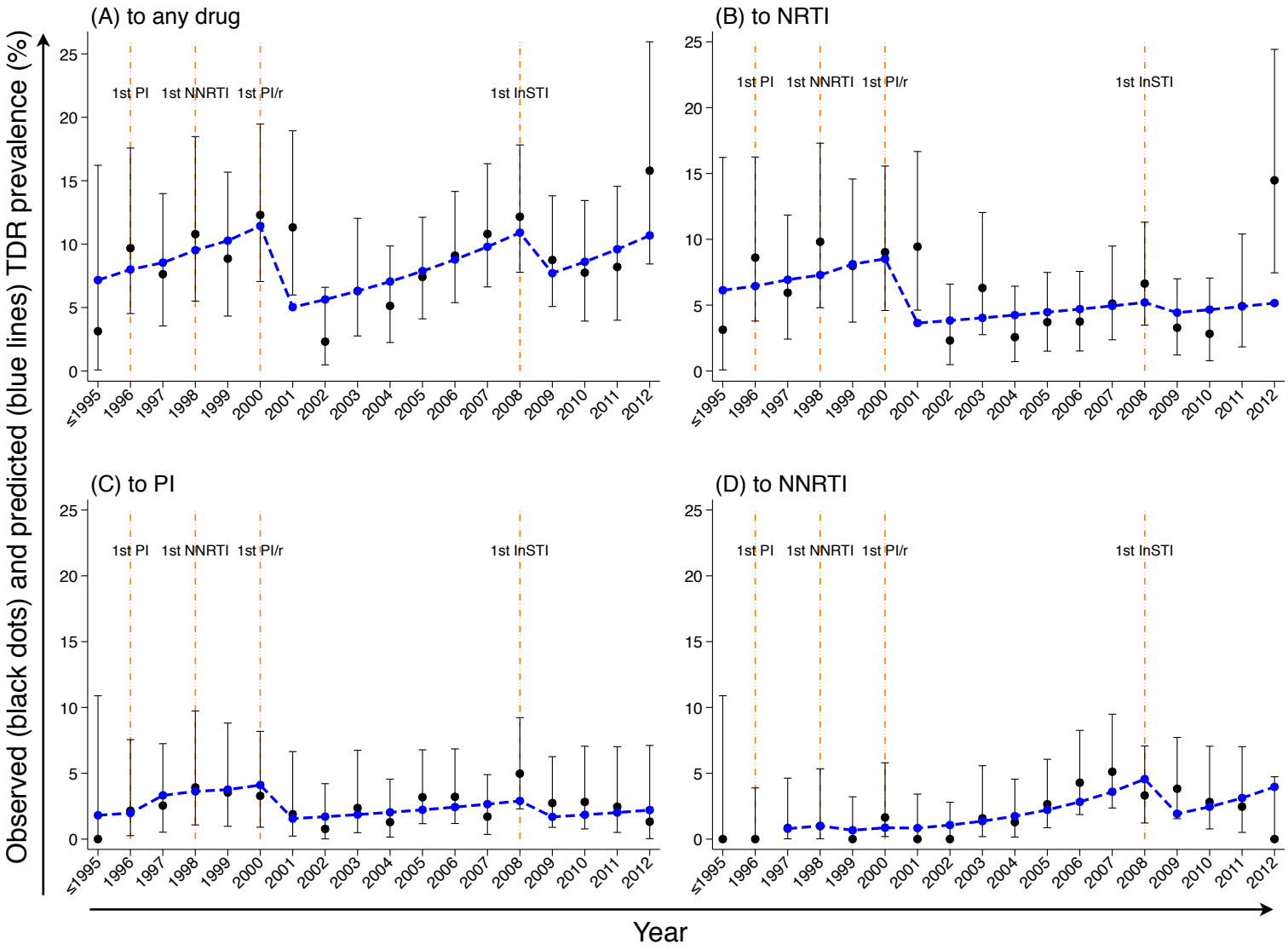
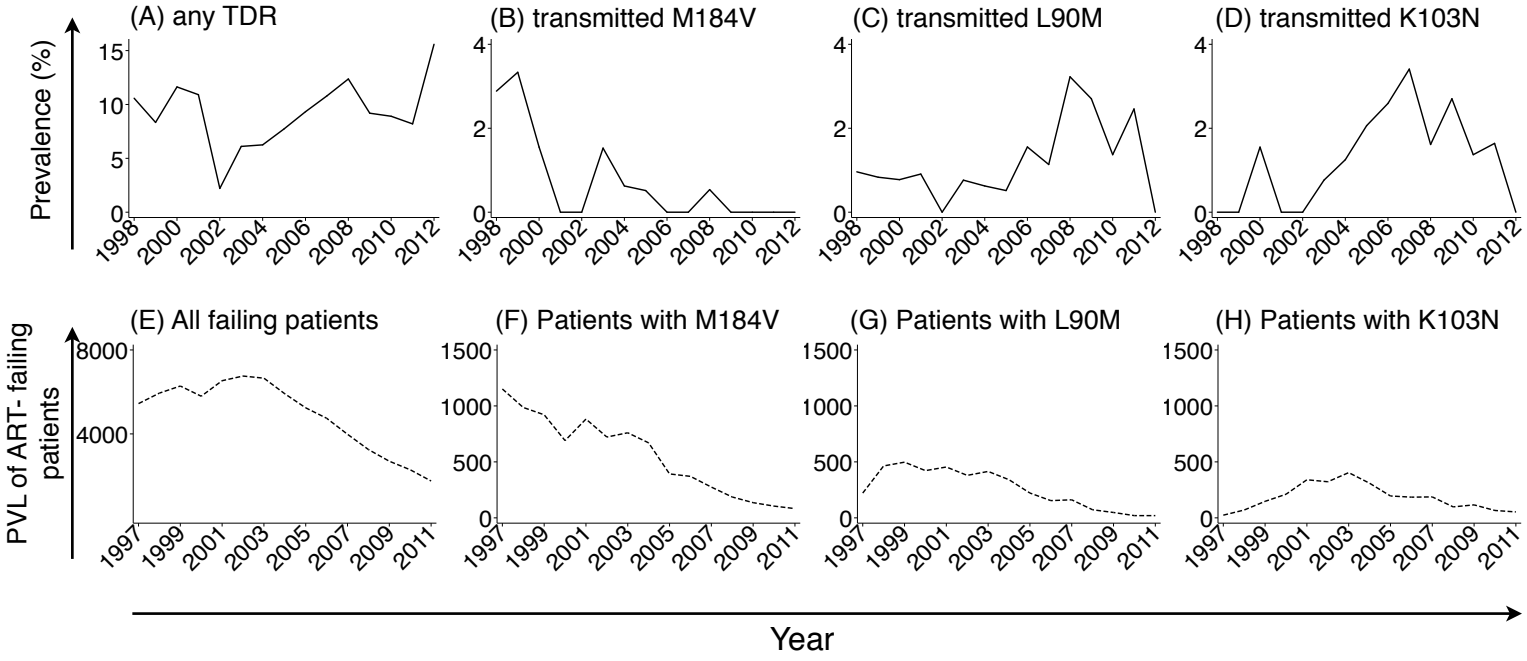


Figure 3



Year	≤ 95	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012
observed, n=2421	35	96	121	104	120	129	110	136	131	160	194	193	176	186	185	146	122	77
predicted, n=2355	32	93	118	102	113	122	106	130	127	156	189	187	176	181	183	142	122	76

Figure 4



Year	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012
TDR, n=2169	--	104	120	129	110	136	131	160	194	193	176	186	185	146	122	77
PVL(all failing patients), n=18097	1421	1528	1580	1437	1619	1631	1592	1411	1250	1149	986	813	668	570	442	--
PVL (failing patients with M184V), n=2159	292	253	236	179	233	185	195	176	100	94	74	52	38	30	22	--
PVL (failing patients with L90M), n=925	50	107	111	99	110	90	101	84	54	38	40	19	12	5	5	--
PVL (failing patients with K103N), n=680	6	15	33	51	82	77	103	76	49	47	48	28	32	18	15	--