Effect of immediate initiation of antiretroviral treatment on the risk of acquired HIV drug resistance

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Objective: We estimated and compared the risk of clinically identified acquired drug resistance under immediate initiation [the currently recommended antiretroviral therapy (ART) initiation strategy], initiation with $CD4^+$ cell count less than 500 cells/μl and initiation with $CD4^+$ cell count less than 350 cells/μl.

Design: Cohort study based on routinely collected data from the HIV-CAUSAL collaboration.

Methods: For each individual, baseline was the earliest time when all eligibility criteria (ART-naive, AIDS free, and others) were met after 1999. Acquired drug resistance was defined using the Stanford classification as resistance to any antiretroviral drug that was clinically identified at least 6 months after ART initiation. We used the parametric g-formula to adjust for time-varying ($CD4^+$ cell count, HIV RNA, AIDS, ART regimen, and drug resistance testing) and baseline (calendar period, mode of acquisition, sex, age, geographical origin, ethnicity and cohort) characteristics.

Results: In 50 981 eligible individuals, 10% had $CD4^+$ cell count more than 500 cells/μl at baseline, and 63% initiated ART during follow-up. Of 2672 tests for acquired drug resistance, 794 found resistance. The estimated 7-year risk (95% confidence interval) of acquired drug resistance was 3.2% (2.8,3.5) for immediate initiation, 3.1% (2.7,3.3) for initiation with $CD4^+$ cell count less than 500 cells/μl, and 2.8% (2.5,3.0) for initiation with $CD4^+$ cell count less than 350 cells/μl. In analyses restricted to individuals with

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baseline in 2005–2015, the corresponding estimates were 1.9% (1.8, 2.5), 1.9% (1.7, 2.4), and 1.8% (1.7, 2.2).

Conclusion: Our findings suggest that the risk of acquired drug resistance is very low, especially in recent calendar periods, and that immediate ART initiation only slightly increases the risk. It is unlikely that drug resistance will jeopardize the proven benefits of immediate ART initiation.

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Introduction

Immediate initiation of antiretroviral therapy (ART) upon diagnosis of HIV infection results in lower risk of serious clinical events [1,2] and virus transmission when compared with delayed ART initiation [3,4]. Therefore, international clinical guidelines now recommend immediate ART initiation for all HIV-positive individuals, regardless of $CD4^+$ cell count [5–8]. A potential concern is that the more prolonged exposure associated with immediate initiation [9] can increase the risk of acquiring drug resistance, which decreases virus susceptibility to specific antiretroviral drugs or drug classes.

Estimates of the long-term risk of acquired drug resistance under immediate ART initiation are needed to fully understand the public health impact of the current guidelines for ART initiation and to estimate the number of individuals in need of second-line therapy. These estimates, however, will need to be obtained from observational studies because the follow-up of the completed randomized trials was relatively short [1,2] and no other large randomized trials are planned. Although several observational studies found that ART initiation at high $CD4^+$ cell counts was associated with a lower risk of drug resistance, [10–13] these studies did not adjust for time-varying confounders and used the date of ART initiation, rather than the date of entry into care, as the time origin.

Here, we estimate and compare the 7-year risks of acquired drug resistance under immediate ART initiation and the previously recommended $CD4^+$ cell count-based initiation strategies. To do so, we use data on HIV-positive individuals receiving HIV care in five European countries.

Methods

Study population

The HIV-CAUSAL collaboration is a consortium of prospective HIV cohorts from Europe and the America. All cohorts record routinely collected data in clinical practice within settings with universal access to care. Data collected include patient characteristics (age, sex, geographical origin, and transmission category), use of ART (type of regimes and dates of start and discontinuation), $CD4^+$ cell counts, and plasma HIV RNA, AIDS-defining conditions, and deaths. Each cohort submits data in a standardized format (http://www.hicdep.org/) to the coordinating center. Ethics approval was granted by the ethics committees of each of the participating cohorts according to country-specific regulations.

The analyses presented here are based on data pooled in September 2015 and were conducted on six cohorts within the collaboration that contributed data on genotypic drug-resistance testing conducted as part of routine clinical care (AMACS from Greece, AIDS Therapy Evaluation in the Netherlands, CoRIS from Spain, Swiss HIV Cohort Study, UK CHIC/UK HIV Drug Resistance Database, and UK Register of HIV Seroconverters from the United Kingdom). The analyses were restricted to individuals who met the following eligibility criteria after 1999: age at least 18 years and $CD4^+$ cell count and HIV RNA measurements within 3 months of each other while AIDS free and ART naive. Because in the early antiretroviral era ART combinations were suboptimal and the drug-resistance testing uncommon, we also restricted to individuals who entered a cohort on or after 1 January 2000. Follow-up started at baseline, defined as the earliest date that all eligibility criteria were met, and ended at the earliest of detection of acquired drug resistance, death, 12 months after the most recent HIV RNA or $CD4^+$ cell count laboratory measurement, cohort-specific administrative censoring, date of pregnancy when known, or initiation of an ART combination not defined as ART (see below).

Treatment strategies

We defined initiation of combined ART as initiation of combination of antiretroviral drugs including at least two nucleoside reverse transcriptase inhibitors (NRTIs) and either one or more protease inhibitors, one nonnucleoside reverse transcriptase inhibitor (NNRTI), one entry/fusion inhibitor, or one integrase inhibitor. We compared the following strategies: immediate ART initiation within 3 months of baseline, initiation within 3 months of a $CD4^+$ cell count less than 500 cells/μl or an AIDS

diagnosis, and initiation within 3 months of a $CD4^+$ cell count less than 350 cells/μl or an AIDS diagnosis [14].

Clinical guidelines recommend drug resistance testing at all episodes of virological failure in ART-treated people [5–8,15]. However, in clinical practice these tests are not always performed. Therefore, to ensure that our estimates reflect the real-world frequency of both drug resistance testing and clinically identified acquired drug resistance, none of the strategies imposed a drug resistance test at virological failure. In sensitivity analyses (see below), we considered an alternative strategy that imposed a drug resistance test at each episode of virological failure.

Drug resistance

The outcome was clinically identified, acquired drug resistance up to 7 years after baseline. Acquired drug resistance was defined as predicted intermediate or high-level resistance to any of the following antiretroviral drugs in use during the study period: atazanavir, darunavir, fosamprenavir, indinavir, lopinavir, nelfinavir, saquinavir, tipranavir (protease inhibitors); lamivudine, emtricitabine, abacavir, didanosine, tenofovir, stavudine, zidovudine (NRTI); nevirapine, efavirenz, etravirine, rilpivirine (NNRTI). Resistance to Integrase Inhibitors (INI) was not examined because it was collected only in one cohort and even in this cohort it was very rare [16]. Predicted resistance was derived using the Genotypic Resistance Interpretation Algorithm, version 7.0 (HIVdb Program, Stanford University, http://hivdb.stanford.edu). To minimize the inclusion of transmitted drug resistance in the outcome definition, mutations conferring resistance to a drug class the person had never taken or genotypic tests on blood samples collected within 6 months after ART initiation were not considered as acquired drug resistance.

Statistical methods

Because standard statistical methods cannot appropriately adjust for time-varying confounders affected by prior treatment [17,18], we used the parametric g-formula to adjust for the time-varying confounders $CD4^+$ cell count, HIV RNA level, AIDS, drug-resistance testing, and treatment class (NNRTI versus non-NNRTI-based regime) as well as for time-fixed confounders measured at baseline.

The parametric g-formula, a generalization of standardization for time-varying treatments and confounders, [18–20] estimates the risk of drug resistance that would have been observed if all patients in the study had adhered to a particular treatment initiation strategy and none had been lost to follow-up, under the assumptions of no residual confounding, no measurement error, and no model misspecification [19,21]. The estimation procedure for the HIV-CAUSAL collaboration has been described elsewhere [20]. Briefly, the procedure has two steps. First, parametric regression models are used to estimate the joint distribution of the outcome, treatment, and time-varying covariates conditional on previous treatment and covariate history. Second, a Monte Carlo simulation using the above estimates is run to simulate the distribution of the postbaseline outcomes and time-varying covariates separately under each ART initiation strategy.

For the first step, we fit separate binary logistic regression models for time-varying indicators of genotypic testing, detection of an acquired drug resistance after a test, death, AIDS-defining condition, ART initiation, measurement of $CD4^+$ cell count, measurement of HIV RNA, and linear regression models for $CD4^+$ cell count and HIV-RNA on the natural logarithm scale. All regression models included the following covariates: the most recent value of these time-varying variables using cubic splines, time since last $CD4^+$ cell count and HIV-RNA measurements, and the following baseline variables: $CD4^+$ cell count (<100, $100-199$,$200-349$,$350-499$, ≥ 500 cells/μl), HIV RNA level ($<10\,000$,$10\,000-100\,000$, $>100\,000$ copies/ml), age (<35,$35-49$,≥ 50 years), sex, mode of HIV acquisition (heterosexual, homo/bisexual, IDUs, or other/unknown), calendar year (2000–2004, 2005–2010, 2011–2015), testing for transmitted drug resistance (i.e., testing done before ART initiation and within 12 months of baseline), geographical origin (Western countries, Sub-Saharan Africa, other, unknown), ethnicity (White, Black, unknown), and cohort. All models also included an interaction term for number of months since ART initiation. The g-formula estimates of risk in the presence of competing risks (death) can be interpreted as an extension of the subdistribution cumulative incidence function to the setting of time-varying treatments and confounders [19]. We used a nonparametric bootstrap procedure based on 500 samples to obtain percentile-based 95% confidence intervals (CIs).

Like all regression-based methods, the parametric g-formula relies on correct model specification. To explore the validity of our parametric assumptions, we compared the observed means of the outcome and time-varying covariates with those predicted by our models. The time-varying means predicted by our models under observed ART initiation were similar to the observed means in the original data (Appendix Figure, http://links.lww.com/QAD/B186). All analyses were conducted with the publicly available SAS macro g-formula (http://www.hsph.harvard.edu/causal/software/).

We conducted subgroup analyses in individuals with baseline $CD4^+$ more than 500 cells/μl (because treatment effectiveness may depend on the initial $CD4^+$ cell count), individuals with baseline date at or after 1 January 2005 (because some study participants started antiretroviral combinations different from those in current use), and individuals originating from Sub-Saharan Africa (because patterns of acquired drug resistance and testing might differ by geographical origin [22] because of different HIV subtype and patterns of treatment adherence).

Sensitivity analyses

The uptake of drug-resistance testing was relatively low in our cohorts, even though HIV guidelines during the study period recommended testing at every episode of virological failure. Although this low uptake is consistent with previous reports from high-income countries [23], it may be partly explained by under ascertainment of drug-resistance testing in our cohorts. We, therefore, conducted two analyses to explore the sensitivity of our results to underascertainment. First, we estimated the 7-year risk of acquired drug resistance if a drug-resistance test had been conducted at each episode of virological failure. Second, we estimated the 7-year risk of drug resistance under the assumption that acquired drug resistance was present at every episode of virological failure. Virological failure was defined as the second of two consecutive HIV RNA at least 400 copies/ml measured at least 6 months after ART initiation [7] preceded by a HIV RNA50 copies/ml or less.

Our estimates would be confounded if the decision to test for drug resistance depended on treatment adherence, a type of information that is usually not captured in HIV cohort data. We, therefore, compared the estimates with and without adjustment for time-varying, self-reported adherence (never missed a dose in the previous 4 weeks versus missed at least one dose) in the Swiss HIV Cohort Study, the only cohort collecting this information longitudinally [24,25].

Finally, to examine the impact of heterogeneity by cohort in the patterns of drug-resistance testing and the collection of genotyping resistance, we reran the analyses excluding each of the six cohorts one at a time.

Results

Table 1 shows the baseline characteristics of the 50 981 eligible individuals: 80% were men, 71% started follow-up after 2004; median [interquartile range (IQR)] $CD4^+$ cell count and age at baseline were 405 (256, 580) cells/μl and 35 (29, 42) years, respectively. 18 002 (25%) individuals had a $CD4^+$ cell count more than 500 cells/μl at baseline. A

Table 1. Baseline characteristics of the 50 981 included individuals, HIV-CAUSAL collaboration 2000–2015.

Baseline characteristics	Eligible	Initiators of ART (%)	Median (IQR) follow-up time, years
$CD4^+$ cell count (cells/μl)			
<100	4021 (8%)	86	3.8 (1.9, 6.8)
100–200	4820 (9%)	86	4 (2.0, 6.8)
200–350	11 581 (23%)	76	3.9 (2.0, 6.5)
350–500	12 557 (25%)	60	3.7 (1.9, 6.0)
>500	18 002 (35%)	44	3.3 (1.7, 5.6)
HIV RNA (copies/ml)			
<10 000	14 491 (28%)	48	3.5 (1.8, 5.9)
10 000–100 000	22 670 (44%)	64	3.8 (1.9, 6.3)
>100 000	13 820 (27%)	77	3.7 (1.8, 6.4)
Sex			
Male	40 933 (80%)	63	3.7 (1.9, 6.2)
Female	10 048 (20%)	62	3.5 (1.8, 6.3)
Age (years)			
<35	25 001 (41%)	57	3.3 (1.8, 5.8)
35–50	21 015 (10%)	67	3.9 (2.1, 6.5)
>50	4965 (31%)	75	3.7 (2.0, 6.3)
Mode of HIV acquisition			
Heterosexual	15 743 (31%)	65	3.7 (1.8, 6.3)
Homo/bisexual	29 482 (58%)	63	3.8 (2.0, 6.3)
IDU	2279 (4%)	54	2.5 (1.3, 5.4)
Other/unknown	3477 (7%)	59	3.3 (1.8, 5.5)
Calendar year			
2000–2004	14 894 (29%)	61	6.7 (3.3, 9.8)
2005–2009	19 254 (38%)	67	4.5 (2.7, 6.0)
2010–2015	16 833 (33%)	59	1.9 (1.2, 2.8)
Region of origin			
Western countries	19 751 (39%)	68	3.8 (2.0, 6.3)
Sub-Saharan Africa	2233 (4%)	66	3.7 (1.8, 6.2)
Rest of the world	4407 (9%)	61	3.3 (1.8, 5.8)
Unknown	24 590 (48%)	59	3.6 (1.8, 6.3)
Ethnicity			
White	20 714 (41%)	63	3.9 (2.0, 6.7)
Black	7991 (16%)	59	3.4 (1.8, 5.9)
Other/unknown	22 276 (44%)	64	3.5 (1.8, 5.9)
Testing for transmitted drug resistance			
No	28 820 (56%)	63	3.4 (1.8, 6.5)
Yes	22 161 (43%)	63	3.8 (2.1, 6.0)
Overall	50 981 (100%)	62	3.7 (1.9, 6.5)

ART, antiretroviral therapy; IQR, interquartile range.

total of 22 161 individuals (43%) were tested for transmitted drug resistance while ART-naive and within 12 months of baseline; transmitted drug resistance was detected in 6.1% of these tests.

During a follow-up of 204 914 person-years, 31 969 (63%) individuals initiated ART at a median $CD4^+$ cell count of 270 cells/μl (177, 369) and 5 (1, 20) months after baseline. Of these, 3207 (10%) initiated ART with $CD4^+$ cell count more than 500 cells/μl. The most common initial ART combinations were NNRTI and two NRTI (64%) and boosted protease inhibitors and two NRTI (29%). Initial combinations containing INI and two NRTI, unboosted protease inhibitors and two NRTI and three NRTI were uncommon (3, 3, and 1%, respectively). The presence of acquired drug resistance was tested for in 2672 samples with median (IQR) HIV RNA of 2475 (236, 33 434) copies/ml. Factors associated with higher rates of testing were female sex, heterosexual HIV transmission group, IDU, and younger age at baseline (Table 2). The rate of drug-resistance testing declined steadily over time. Similar associations and trends by baseline characteristics and calendar period were found for the rates of detection of drug resistance and of virological failure (Table 2). Cohorts differed in the rates of drug resistance testing and of virological failure (Appendix Table 1, http://links.lww.com/QAD/B186).

There were 1874 episodes of virological failure, of which 617 (33%) were followed by a drug-resistance test within 12 months. The proportion of episodes of virological failure followed by a test increased from 20% in 2000–2004 to 37 and 36% in 2005–2009 and 2010–2015, respectively. Resistance to any drug was detected in 794 (30%) samples. Resistance to NNRTI, NRTI and boosted protease inhibitors was detected in 512 (19%), 576 (22%), and 67 (3%) samples, respectively.

The observed risk of acquired drug resistance was 2.7% at 7 years after baseline. The estimated 7-year risk (95% CI) of

Table 2. Tests for acquired drug resistance and number of episodes of virological failure[a] and corresponding rates per 1000 person-years 6 months after antiretroviral therapy initiation.

	Tests for acquired drug resistance		Tests detecting acquired drug resistance		Virological failure episodes[a]	
Baseline characteristics	N	Tests/1000 person-years	N	Tests/1000 person-years	N	Episodes/1000 person-years
Overall	2672	20.1	794	6	1874	14.1
$CD4^+$ cell count (cells/μl)						
<100	449	28.9	207	13.3	227	14.8
100–200	433	23.5	138	7.5	254	13.8
200–350	750	19.3	216	5.6	546	14.1
350–500	487	15.8	111	3.6	403	13
>500	553	19.1	122	4.2	404	15.3
HIV RNA (copies/ml)						
<10 000	435	16	111	4.1	425	15.6
10 000–100 000	1117	18.4	338	5.6	803	13.2
>100 000	1120	25	345	7.7	646	14.4
Sex						
Male	1959	18.1	574	5.3	1300	12
Female	713	29	220	9	574	23.4
Age (years)						
18–35	1318	23.9	382	6.9	953	17.3
35–50	1136	18.3	351	5.7	790	12.8
>50	218	14	61	3.9	131	8.4
Mode of HIV acquisition						
Heterosexual	1087	25.8	367	8.7	778	18.7
Homo/bi-sexual	1326	16.9	337	4.3	864	11
IDU	127	30.1	36	8.5	120	28.5
Other/unknown	132	17	54	7	102	13.2
Calendar year						
2000–2004	1403	24	486	8.3	1104	18.9
2005–2009	1069	19	264	4.7	609	10.8
2010–2015	200	11.1	44	2.4	162	9
Region of origin						
Western countries	524	9.2	181	3.2	602	10.6
Sub-Saharan Africa	159	28.2	65	11.5	128	22.7
Rest of the world	137	13.6	55	5.4	109	10.8
Unknown	1852	30.9	493	8.2	1035	17.2
Ethnicity						
White	1288	22.1	312	5.4	849	14.6
Black	727	38.1	245	12.8	461	24.2
Other/unknown	657	11.9	237	4.3	564	10.2
Testing for transmitted drug resistance						
No	1478	19	508	6.6	1200	15.5
Yes	1194	11.1	286	5.2	674	12.2

HIV-CAUSAL collaboration 2000–2015.
[a]Virological failure was defined as the second of two consecutive HIV RNA ≥400 copies/ml preceded by HIV RNA ≤50 copies/ml.

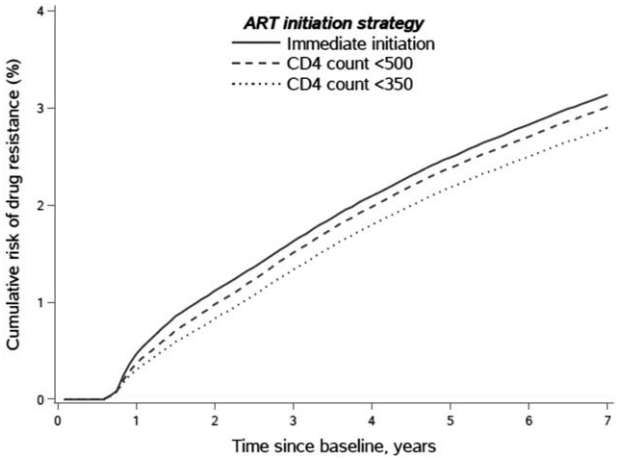

Fig. 1. Risk of acquired drug resistance up to 7 years after baseline by ART initiation strategy. ART, antiretroviral therapy. HIV-CAUSAL collaboration 2000–2015.

acquired drug resistance was 3.2% (2.8, 3.5) for immediate ART initiation, 3.1% (2.7, 3.3) for initiation at $CD4^+$ less than 500 cells/μl, and 2.8% (2.5,3.1) for initiation at $CD4^+$ less than 350 cells/μl. Compared with immediate initiation, the risk difference (95% CI) was −0.13% (−0.22, −0.06) under initiation at $CD4^+$ less than 500 cells/μl, and −0.37% (−0.52, −0.22) under initiation at $CD4^+$ less than 350 cells/μl (Fig. 1 and Table 3).

When restricting the analyses to individuals with baseline in 2005–2015, the estimated 7-year risk (95% CI) of acquired resistance was 1.9% (1.8, 2.5) for immediate ART initiation, 1.9% (1.7,2.4) for initiation at $CD4^+$ less than 500 cells/μl, and 1.8% (1.7,2.2) for initiation at $CD4^+$ less than 350 cells/μl. When the analyses were restricted to individuals with baseline $CD4^+$ more than 500 cells/μl the 7-year risk estimates were 1.6% (1.2, 2.3), 1.9% (1.4, 2.4), and 1.6% (1.2,2.1) for immediate initiation and initiation with $CD4^+$ cell count less than 500 cells/μl and $CD4^+$ cell count less than 350 cells/μl, respectively. Under immediate ART initiation the estimated 7-year risk of acquired drug resistance was 4.4% (3.8, 5.0) when we imposed a drug-resistance test at each episode of virological failure, and 10.9% (10.1, 11.4) when we assumed that every instance of virological failure was because of acquired drug resistance (Table 4). Results did not materially change in the subgroup analyses sequentially excluding each of the cohorts (Appendix Table 2, http://links.lww.com/QAD/B186). In the Swiss HIV Cohort Study data, the estimates of risk under all strategies adjusting for self-reported adherence were similar to the estimates not adjusting for adherence (risk difference <0.4%).

Discussion

In HIV-positive individuals receiving routine clinical care in Europe, we estimated that the risk of acquired drug resistance was similar under immediate and delayed ART initiation. Compared with ART initiation with $CD4^+$ less than 500 cells/μl or AIDS and $CD4^+$ less than 350 cells/μl or AIDS, immediate ART initiation increased the 7-year risk of acquired drug resistance by only 0.13 and 0.37%, respectively. The estimated 7-year risk of clinically identified acquired drug resistance was approximately 3% under all ART initiation strategies. These risks and risk differences were even lower in individuals with initial $CD4^+$ cell count more than 500 cells/μl and individuals who entered the study after 2004.

To our knowledge, this is the first study to estimate the public health impact of immediate ART initiation on the risk of acquired drug resistance. Previous studies found that higher $CD4^+$ cell count at ART initiation was associated with lower risk of resistance [10–13]. Unlike in these studies, we quantified the risk of drug resistance since baseline, a proxy of entry into HIV care, rather than ART initiation. Our estimates of risk take into account that immediate initiation implies longer exposure to ART and thus increased chance of drug resistance and the risk of acquiring drug resistance before ART initiation is zero. Our findings indicate that it is unlikely that the clinical benefits of early ART initiation demonstrated by

Table 3. Seven-year risk of acquired drug resistance and risk difference by antiretroviral therapy initiation strategy and inclusion criteria.

Inclusion criteria	ART initiation strategy	Risk at 7 years, % (95% CI)	Risk difference (95% CI)
All patients ($N = 50\,981$)	Immediate	3.2 (2.8, 3.5)	0 (Ref.)
	<500 cells/μl	3.1 (2.7, 3.3)	−0.13 (−0.22, −0.06)
	<350 cells/μl	2.8 (2.5, 3.0)	−0.37 (−0.52, −0.22)
Baseline in calendar years 2005–2015 ($N = 36\,087$)	Immediate	1.9 (1.8, 2.5)	0 (Ref.)
	<500 cells/μl	1.9 (1.7, 2.4)	−0.08 (−0.21, 0.04)
	<350 cells/μl	1.8 (1.7, 2.2)	−0.15 (−0.39, 0.02)
Baseline $CD4^+$ cell count >500 cells/μl ($N = 18\,002$)	Immediate	1.6 (1.2, 2.3)	0 (Ref.)
	<500 cells/μl	1.9 (1.4, 2.4)	0.22 (−0.18, 0.48)
	<350 cells/μl	1.6 (1.2, 2.1)	−0.08 (−0.62, 0.36)
Individuals with Sub-Saharan Africa as region of origin ($N = 2233$)	Immediate	6.5 (4.2, 9.3)	0 (Ref.)
	<500 cells/μl	6.2 (4.2, 9.1)	−0.27 (−0.85, 0.11)
	<350 cells/μl	5.6 (3.7, 8.7)	−0.90 (−1.67, 0.00)

HIV-CAUSAL collaboration 2000–2015. ART, antiretroviral therapy; CI, confidence interval.

Table 4. Sensitivity analyses to examine the role of potential underascertainment of drug resistance testing. Estimated 7-year risk of acquired drug resistance and risk difference by antiretroviral therapy initiation strategy.

Outcome	ART initiation strategy	Risk at 7 years, % (95% CI)	Risk difference (95% CI)
Acquired drug resistance under the observed data	Immediate	3.2 (2.8, 3.5)	0 (Ref.)
	<500 cells/μl	3.1 (2.7, 3.3)	−0.13 (−0.22, −0.06)
	<350 cells/μl	2.8 (2.5, 3.0)	−0.37 (−0.52, −0.22)
Acquired drug resistance assuming a drug resistance test at every virological failure[a] episode	Immediate	4.4 (3.8, 5.0)	0 (Ref.)
	<500 cells/μl	3.8 (3.3, 4.3)	−0.60 (−0.71, −0.44)
	<350 cells/μl	3.1 (2.8, 3.6)	−1.27 (−1.47, −0.99)
Virological failure[a] episode	Immediate	10.9 (10.1, 11.4)	0 (Ref.)
	<500 cells/μl	9.3 (8.6, 9.8)	−1.58 (−1.74, −1.39)
	<350 cells/μl	7.5 (6.9, 7.8)	−3.42 (−3.69, −3.07)

HIV-CAUSAL collaboration 2000–2015. ART, antiretroviral therapy; CI, confidence interval.
[a]Virological failure was defined as the second of two consecutive HIV RNA ≥400 copies/ml preceded by HIV RNA ≤50 cells/μl.

randomized clinical trials [1,2] and observational studies [26–30] will be lessened by development of acquired drug resistance. In high-income countries, the need for second-line treatments because of the development of acquired drug resistance will increase only slightly under the new ART initiation guidelines [5–8]. In particular, our findings indicate that in a hypothetical cohort of 1000 patients, immediate initiation would imply only four additional cases in need of second-line treatment over a 7-year period compared to initiation with $CD4^+$ cell count less than 350 cells/μl or AIDS. Moreover, because of the increasing use of antiretroviral treatments associated with low drug resistance such as INI and new generation protease inhibitors, which were underrepresented in our data, we expect this increase to be even lower in the longer term. Further, the low risk of acquired drug resistance after 2004 indicates that acquired drug resistance is becoming a rare phenomenon in Europe, though higher among individuals born in Sub-Saharan Africa. This is compatible with previous findings from the Swiss HIV Cohort Study [22,31] and generalizes them to other European countries with similar access to health care.

As in previous studies aimed at estimating the comparative effectiveness of immediate ART initiation [27], we analyzed the data both including all patients regardless of initial $CD4^+$ cell count (to estimate the public health impact) and including only patients with high $CD4^+$ cell count at baseline (to estimate the effectiveness in this particular subgroup). The low risk of drug resistance in the subgroup of individuals with baseline $CD4^+$ cell count more than 500 cells/μl alleviates the concern that individuals who are diagnosed with HIV while asymptomatic might be less likely to adhere to treatment.

International and national HIV guidelines recommend testing for transmitted drug resistance in all ART-naïve individuals and testing for acquired drug resistance in ART-treated people after all episodes of virological failure. Despite these recommendations only 43% of the people included in our study were known to be tested for transmitted drug resistance and only 33% of virological failure episodes were known to be followed by an acquired drug resistance test. Our estimates were, however, robust under different scenarios of potential underascertainment of acquired drug resistance.

The main strengths of our study are the large sample size of over 50 000 individuals and the setting in HIV clinics in Europe that are considered representative of routine clinical practice [32]. Care should be taken with generalizing our results to resource-limited settings because of the differences in the HIV epidemics, and availability of viral load monitoring, of drug-resistance testing facilities, and of second and third-line therapy.

This study has several limitations. First, the validity of our estimates relies on the assumption that all factors that influenced the decision to initiate ART and the risk of developing drug resistance were adjusted for. We expect this assumption to approximately hold because we adjusted for the most important factors used to decide whether to initiate ART such as $CD4^+$ cell count, HIV RNA, and AIDS. Second, our methods require all models to be correctly specified. This condition cannot be guaranteed, but it seems plausible because our models resulted in simulated datasets with distributions of outcome and time-varying covariates similar to those in the original data. Third, we could not estimate the long-term effect of immediate initiation because approximately only 25% of included patients had follow-up longer than 7 years. Fourth, given that only half of the patients were genotyped before ART initiation, some resistance-associated mutations may, in fact, have been transmitted. It is, therefore, possible that our estimates of risk of acquired drug resistance are somewhat pessimistic. Finally, only a small proportion of patients initiated treatment with INI, an ART regimen currently recommended as first-line therapy, therefore, we could not appropriately assess drug resistance for this drug class. However, according to recent reports, drug resistance to INI is very rare and INI-treated patients exhibit low rates of virological failure [16,31].

In conclusion, the risk of acquired drug resistance after immediate ART initiation, the currently recommended strategy for ART initiation, is very small and not

materially larger than if treatment is deferred. Therefore, it is unlikely that the benefits of immediate ART initiation will be compromised by development of acquired drug resistance in high-income countries and that immediate initiation will imply a substantial increase in need of second-line treatments. Continual efforts should be made to monitor trends in transmitted drug resistance and acquired drug resistance in high and low-income countries.

In summary, we estimated and compared the risk of acquired drug resistance under immediate initiation (the currently recommended ART initiation strategy) and $CD4^+$ cell count-based strategies. Immediate initiation increased the risk of drug resistance, but the magnitude of the change were very small.

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Conflicts of interest

There are no conflicts of interest.

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