Incidence and risk factors for liver enzyme elevation among naive HIV-1 infected patients receiving antiretroviral therapy in ICONA Cohort

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Running title: LEE in first line ART

Synopsis

Objectives: To evaluate the incidence and risk factors for liver enzyme elevations (LEE) in patients initiating first-line antiretroviral therapy (ART) in ICONA prospective observational cohort, between June 2009 and December 2017.

Patients and methods: 6,575 ART-naïve patients were selected, initiating 2 NRTI associated to a boosted PI N=2,436 (37.1%), a NNRTI N=2,384 (36.3%), or an integrase inhibithor (INSTI) N=755 (26.7%). Hepatitis B Virus (HBV) surface Antigen and Hepatitis C virus (HCV)-RNA were detected in 3.9% and 5.8% of the study population. Inverse probability weighted Cox regression analysis was used to calculate the hazard ratios (HR), according to first-line regimen, for LEE, defined as ALT or AST increases of ≥ 2.5 x ULN (upper limit of normal) for patients with normal baseline values or ≥ 2.5 x baseline, for patients with higher baseline values.

Results: One hundred eighty-three LEE occurred over 20,722 patient years follow up. After adjusting for main confounders, the risk of LEE resulted halved with INSTI compared to NNRTI (HR 0.46, 95%CI 0.25-0.86), with a significant reduction in raltegravir group (HR 0.11, 95%CI 0.02-0.84 using NNRTI class as reference). HR for LEE resulted significantly higher in subjects with HBV or HCV coinfection, in patients with poorly controlled HIV infection and in those who acquired HIV through homosexual transmission.

Conclusions: In our study, INSTI use almost halved the risk of LEE compared to other regimens. This datum could be particularly important for choosing ART in patients with risk factors for liver toxicity such as HCV and HBV co-infections.

Introduction

Liver enzyme elevations (LEE) are common among patients infected with HIV and recognize multifactorial etiologies, often, although not invariably, linked to Hepatitis B (HBV) or C (HCV) co-infection. [1,2] Although LEE arising from antiretroviral-associated liver injury are now less frequently observed than in the past, [3] their occurrence may influence the clinical management and the choice treatment in people living with HIV (PLWHIV), as high ALT levels have been associated with higher all-cause mortality, [4] while severe AST elevation during the course of combined ART has been associated with poor survival. [5] The possibility of a drug toxicity as underlying cause of LEE should always be investigated, and is often suspected, especially in previously untreated patients who develop LEE after starting their first line ART. Many molecules have been linked to possible LEE events in the past, but few data are available on new drug classes such as integrase strand transfer inhibitors (INSTI), especially in the clinical post-marketing phase. [6-8] Albeit INSTI use has ben associated with low risk of LEE in clinical trials, [9-17] some few reports of liver toxicity have emerged last years. [7,18-20] Moreover, few data about INSTI tolerability are

available in the population of PLWHIV co-infected with HBV or HCV, especially in comparison with other classes of antiretroviral agents. [21,22] The aim of the present study is to compare the incidence of LEE events in PLWHIV in first line antiretroviral therapy with different class agents, and identify the possible predictors of LEE.

Materials and methods

The Italian Cohort Naive Antiretrovirals Foundation Study (ICONA) is a multi-centre, prospective and observational study cohort, recruiting ART-naïve PLWHIV from the outset, in 1997. ICONA study has been approved by Institutional Review Boards of all the participating centres. To date, the cohort enrolled more than 17,000 patients; 52 centers from 37 cities throughout Italy, are currently in operation (further information on www.fondazioneicona.org). ICONA collects data starting from the date of entry in the cohort, from ART naïve, till last available follow-up. All patients aged ≥ 18 years old who receive a new diagnosis of HIV infection and who are in care in Infectious Diseases Hospital Units participating to ICONA, are consecutively asked to participate to the cohort. Patients are subsequently enrolled, if they agree to participate and sign consent forms, in accordance with the ethical standards of the committee on human experimentation and the Helsinki Declaration (1983 revision). Demographic, clinical, laboratory data and information on therapies are collected and recorded online (www.icona.org); sensitive data are collected only in anonymous form. Details of the cohort have been described elsewhere.[23] Subjects enrolled in the ICONA cohort are generally representative of the italian HIV-diagnosed population.[24]

We included in the present analysis all PLWHIV who initiated their first antiretroviral regimen since January 1st 2009, with 2 nucleoside reverse transcriptase inhibitors (NRTI) plus a ritonavir/cobicistat-boosted protease inhibitor (PI/b), a non-nucleoside reverse transcriptase inhibitor (NNRTI) or an INSTI.

Patients were included if they were naïve to ART, and had available AST or ALT value both at baseline and at least at one following evaluation after ART initiation. Supplementary Table 1

contains the comparison of baseline characteristics of 6575 patients selected for the study and 2048 patients excluded who started ART after 1st Jan 2009.

LEE was defined by a rise of ALT or aspartate aminotransferase (AST) of grade ≥ 2, i.e. ≥ 2.5 x ULN (upper limit of normal) for patients with baseline ALT in the normality range or ≥ 2.5 x baseline value, if the baseline value was higher than ULN. [25] Grade 3 LEE was defined as ALT or AST rise to > 5 x ULN for patients with baseline ALT or AST in the normality range or > 3.5 x baseline value, if the baseline value was higher than ULN; grade 4 LEE was defined as ALT or AST rise to > 10 x ULN for patients with baseline ALT or AST in the normality range or > 5 x baseline value, if the baseline value was higher than ULN. [25] We considered ALT and AST ULN 19 U/L in females and 30 U/L in males. [26]

Data collection

Major confounding variables which were controlled for analytically included: mode of HIV transmission (heterosexual or homosexual contacts, intravenous drug use, other/unknown), HBV coinfection (defined by HBsAg positive, negative, not tested), and HCV coinfection (combination of HCVAb positive/negative or not tested and HCV-RNA viral load detectable, undetectable or not tested), use of tenofovir/emtricitabine as backbone. Other time-varying covariates were included both at baseline and during follow-up: CD4+ (in classes: 0-199, 200-350, 351-500, >500 cells/mm^3) and HIV-RNA (at baseline: <5, >5 $\log_{10}$ copies/mL; current: not detectable, detectable ≤50 copies/mL, detectable >50 copies/mL), alcohol use, presence/absence of concurrent medications (any drugs other than ART), presence/absence of comorbidity.

Alcohol consumption is collected by physician interview at study enrolment and at subsequent clinical visits (at least every 6 months) during follow-up. Exact questions in the patients' interview (with possible responses) are as follows: 1) Do you currently drink alcohol? (Yes/No/Unknown); 2) How frequently do you drink alcohol? (Daily/Non-daily/Unknown); 3) How many units of (Wine/Beer/Spirits) do you consume per day? Frequency and quantity of units of drink consumed

are translated into drinking categories by mapping the data to the definitions described in the EASL guidelines. [27]

Presence of comorbidity was defined if at least one of the following conditions was present: cardiac or cerebrovascular events (myocardial infarction, coronary bypass, coronary angioplasty, carotid endarterectomy, stroke, cerebral hemorrhage), bacterial meningitis, renal disease (chronic kidney disease, defined by at least two consecutive GFR <60 mL/min per 1.73 m^2, estimated by CKD-EPI formula; or dialysis or renal transplantation) and all non-AIDS defining malignancies. GGT level was defined abnormal if >29 U/L in women, while in men the threshold was defined according to age: >31 for 18-35 years, >35 for 36-40, >37 for 41-45, >39 for 46-50, >42 for 51-54, >45 for 55, >48 for >46.

Statistical Methods

Primary end-point of the analysis was defined as first confirmed grade ≥2 LEE after cART initiation. Secondary end-point was first confirmed grade ≥3 LEE.

Baseline of the analysis was ART initiation and each patient was followed until the occurrence of LEE. In patients who did not experience the outcome, follow up time was censored at last available ALT or AST or death. The analysis was performed according to an intention to treat (ITT) principle so that any change of drug during follow-up was ignored. The incidence rate of LEE was calculated as number of new cases over person-years of follow up at risk.

To take into account that loss to follow-up due to competing events such as death or loss to follow-up for other reasons could be informative, namely the censoring could be associated with the analysis outcome, we calculated inverse probability of censoring weights by means of two sets of logistic regression models. Then, to estimate the causal hazard ratio of LEE according to different third drug class started in the first ART regimen we fitted a weighted pooled logistic regression (which amounts to a weighted Cox regression model) adjusting for main confounding variables including both time-fixed and time-varying covariates.

All the analyses were repeated in the subgroup of patients with normal values of baseline GGT.

To evaluate if viral hepatitis status was an effect modifier in the association between risk of LEE and type of ART regimen, the interaction was formally tested.

Ethics

ICONA study has been approved by Institutional Review Boards (IRB) of all the participating centres. The full list of IRB ICONA approvals, including approval reference numbers, is available in supplementary materials.

Results

Overall, 6,575 ART-naïve patients were included, 2,384 (36.3%) initiating 2 NRTI+NNRTI, 2,436 (37.1%) NRTI+PI/b and 1,755 (26.7%) NRTI+INSTI. Patients were 80.8% male, 26% had <200 CD4+ cells/mm^3 at baseline and median age was 39 (32-47) years. HBsAg and HCV-RNA were detected in 3.9% and 5.8% of the study population. The main characteristics of the study population according to drug class started are showed in Table 1.

Incidence and predictors of LEE (grade ≥ 2)

One hundred eighty-three LEE occurred over 20,722 patient years follow up (PYFU) (Incidence rate of LEE= 8.8 x 1000 PYFU, 95%CI 7.6-10.2), 93 events of grade 2 (50.8%), 42 (23.0%) of grade 3 and 48 (26.2%) of grade 4. LEE occurred after a median time of 17 (6-38) months. At univariable analysis patients in treatment with INSTI were less likely to experience LEE than patients treated with NNRTI (HR 0.48, 95%CI 0.27-0.85, Figure 1). After adjusting for main confounders, INSTI-treated still had lower hazard of LEE when compared with patients treated with NNRTI (AHR 0.46, 95%CI 0.25-0.86, Table 2a). Raltegravir resulted the INSTI whose use was associated with the lowest risk of LEE, and the only to maintain significant protective effect at multivariable analysis when single agents of the INSTI class were compare to NNRTI (AHR 0.11,

95%CI 0.02-0.84, Table 2b). No differences were found in patients using tenofovir/emtricitabine as a backbone, as compared to other NRTIs. HR for LEE resulted significantly higher in subjects with HBV or HCV coinfection versus HIV mono infected, and in those with higher current HIV RNA. Table 3 reports the incidence rate of LEE of grade ≥ 2 and ≥ 3 among patients treated with NNRTI, boosted PI and INSTI according to HCV and HBV infection. The interaction between the type of third agent class and the coinfection HCV and HBV was tested. The risk of LEE both of grade ≥ 2 and ≥ 3 was not different according to the status of HCVAb and of HBsAg (positive or negative) for different drug classes for grade ≥ 2 LEE p at interaction test=0.117, p=0.190 respectively, for grade ≥3 LEE p at interaction test=0.590, p=0.870 respectively). The dataset was also reviewed to account for acute hepatitis A (HAV) and hepatitis E (HEV) viral infections at time of LEE. Fourty five patients included in the study experienced acute HAV infection, and in 6 patients LEE and HAV were reported in the same timeframe (between -49 and +45 days from LEE occurrence). All 6 patients were MSM. No cases of acute HEV were reported.

Incidence and predictors of severe LEE grade ≥ 3

LEE of grade ≥ 3 occurred in 90 ART-naive patients over 20,983 PYFU (Incidence rate of severe LEE 4.3 x 1000 PYFU), after a median time of 14 (6-36) months. Predictors of severe LEE were similar to factors associated with LEE of grade ≥2. Risk of severe LEE resulted significantly lower in patients with high current CD4 count (CD4 = 350-500 versus <200 cells/mm^3 HR 0.38, 95%CI 0.15-0.94; CD4>500 cells/mm^3 HR 0.31, 95%CI 0.12-0.76), while the risk was higher in HBV (HR 2.33, 95%CI 1.08-5.04) or HCV co-infected patients (HR 7.70, 95%CI 4.26-13.91), in those with current detectable HIV RNA versus not detectable (HR 2.17, 95%CI 1.09-4.31) and in those who acquired HIV through MSM transmission versus heterosexual (HR 2.61, 95%CI 1.49-4.57, p=.001). Type of first-line ART or use of tenofovir/emtricitabine as backbone did not influence the incidence of severe LEE (HR for INSTI versus NNRTI 0.64, 95%CI 0.31-1.33; and HR for PI/b versus

NNRTI 0.81, 95%CI 0.49-1.33; HR for TDF/FTC versus other NRTIs 1.44, 95%CI 0.55-2.86, Supplementary Table 2).

Incidence and predictors of LEE in patients with normal GGT at baseline

Four thousand one hundred eighteen patients had normal GGT at cART initiation. In this subgroup of patients (N=4118) 114 LEE occurred over 13,182 PYFU, 61 events of grade 2 (53.5%), 22 (19.3%) of grade 3 and 31 (27.2%) of grade 4. Incidence rate of grade ≥ 2 LEE was 8.6 x 1000 PYFU (95%CI 7.2-10.4) and of at least grade ≥ 3 LEE was 4.3 x 1000 PYFU (95%CI 3.5-5.3). At univariable and multivariable analysis patients in treatment with INSTI showed a reduced risk of grade ≥ 2 LEE, but not significant, (crude HR 0.42, 95%CI 0.15-1.22; adjusted HR 0.41, 95%CI 0.14-1.22) using patients treated with NNRTI as reference (Supplementary Table 3).

Discussion

In the present study, we investigated the frequency of LEE in a large cohort of patients initiating first line ART. The main result of the study is that INSTI were associated with nearly halved risk of LEE when compared to ART containing PI/b or NNRTI. Indeed, little is known on the liver toxicities of newer ART regimens, beyond clinical trials. [6,19,28,29] In particular, data are still scarce on INSTI, that, altohugh generally well tolerated, [30] still have a relatively short post-commercial follow up. [31-39] The clinical trials performed in naïve patients showed a low rate of ART discontinuations due to LEE, i.e. <1% for raltegravir, elvitegravir and dolutegravir [9-17] and only a very low rate of the adverse events reported by real life studies were indeed hepatic events. [7,18-20] In line with those results, in our study INSTI showed the most favourable hepatic profile, based on LEE events. In particular, raltegravir use was associate to 89% risk reduction of LEE, suggesting a very safe hepatic profile. This finding might be linked to raltegravir methabolism, as raltegravir is the only INSTI to completely by-pass the cytochrome system, thus resulting the one with the lower potential for hepatic toxicity and drug-drug interactions. [29] On the other side, the possibility of liver

toxicity linked to both PI/b and NNRTI exposure is already known, especially with older drugs of both classes. [40-43] However, the differences among antiretroviral classes were not confirmed when analysing only severe LEE (grade ≥3), and the clinical impact of low grade LEE is not fully understood. Previous works reported higher mortality in HIV–infected patients with ALT above the normal value and in those with AST elevation > 200 UI/mL, but the actual implications of LEE are still object of study. [4, 22, 44] However, the occurrence of LEE is an undesirable event that implies, at a minimum, the intensification of scheduled blood examination, diagnostic workout to find LEE causes, and sometimes even therapy discontinuation. [22, 45-47] The long interval found in this study between ART initiation and LEE occurrence (i.e. 17 months), however, makes it difficult to impute LEE exclusively to the toxicity of underlying ART, but may suggest a mechanism of additive effects of multiple factors, wich add up over time, and that may be more or less fauvored by the concomitant ART. Of note, LEE incidence rate in our work was lower than those reported in the past, [21,41,48,49] suggesting a more hepatic friendly profile of the newer therapies, togeter with reduction of hepatic comorbidities such as hepatitis coinfection. [50] Indeed, besides initial ART, the stronger predictors of LEE resulted HBV or HCV coinfections, in accordance with previous studies. [41,51] Also, an uncontrolled HIV infection (lower CD4 count or higher HIV RNA) resulted associated with risk of LEE, as expected based on literature data [25,41] and on the known toxic effects of HIV infection on hepatic cells. [52,53]

Finally, MSM patients resulted at higher risk of severe (grade ≥ 3) LEE. This finding was unexpected [48,54,55] and might have been driven by underlying lifestyles and habits of MSM participating to this study, including recreational chemsex. [56] Moreover, although only six cases of LEE have been reported in patients with concomitant diagnosis of acute HAV infection in our study, we cannot exclude that some additional cases of LEE could have been due to HAV or HEV infection not diagnosed or not reported in ICONA. [57]

The study has several potential limitations. First, due to the observational nature of the study, patients were not randomly assigned to drug therapies, and confounding bias could have occurred, based on clinicians' decisions to prescribe different therapies in patients with different baseline characteristics. One key confounder in the analysis is adherence to treatment as it is clearly a common cause of type of ART prescription and risk of LEE and this variable in unmeasured in the ICONA study. In addition, other potential confounding factors such as the prevalence of recreational habits and illicit substance use was also not taken into account in the analysis. In this regard, it is worth noting that interactions between ART and other substances could also be important drivers of LEE, and could have contributed to show better tolerance profile for INSTIs and have not been investigated. Third, we have chosen the pragmatic approach of comparing the regimens according to the intention to treat principle so this analysis compares the risk of LEE according to the drug class which was initiated rather than the class used at the time of event. Of note, the weighted Cox correctly controls for potential selection bias due to censoring which might have occurred over follow-up.Finally, our ability to detect differences in the risk of grade ≥ 3 LEE hepatotoxicity associated with specific drugs might be limited by the relatively small number of these events. With those limitations, our study has the strenght to analyse a large cohort of patients treated in first line with modern ART regimens. Our study shows a low incidence rate of LEE with modern first line ART and an almost halved risk of LEE in PLWHIV treated with an INSTI compared to other regimens, with raltegravir use associated with the lowest risk. HCV and HBV co-infections remain the major drivers of liver enzyme abnormalities.

Acknowledgements:

The preliminary results of the present study have been previously presented in the poster session of the 10th Italian Conference on AIDS and Antiviral Research (ICAR) 2018.

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Mussini, C Puzzolante (Modena); C Migliorino, G Lapadula (Monza); V Sangiovanni, G Borgia, V Esposito, F Di Martino, I Gentile, V Rizzo (Napoli); AM Cattelan, S Marinello (Padova); A Cascio, M Trizzino (Palermo); F Baldelli, E Schiaroli (Perugia); G Parruti, F Sozio (Pescara); G Magnani, MA Ursitti (Reggio Emilia); M Andreoni, A Antinori, R Cauda, A Cristaudo, V Vullo, R Acinapura, D Moschese, M Capozzi, A Mondi, A Cingolani, M Rivano Capparuccia, G Iaiani, A Latini, R Gagliardini, MM Plazzi, S Savinelli, A Vergori (Roma); M Cecchetto, F Viviani (Rovigo); G Madeddu, A De Vito(Sassari); B Rossetti, F Montagnani (Siena); A Franco, R Fontana Del Vecchio (Siracusa); D Francisci, C Di Giuli (Terni); P Caramello, G Di Perri, S Bonora, GC Orofino, M Sciandra (Torino); M Bassetti, A Londero (Udine); G Pellizzer, V Manfrin (Vicenza); G Starnini, A Ialungo (Viterbo).

Funding: This study was carried out as part of our routine work in the context of ICONA cohort.

Transparency declarations: ICONA Foundation is supported by unrestricted grants from Gilead, Janssen, MSD and ViiV Healthcare.

Contributions: LT and ADB ideated the study and wrote the final version of the paper, PL and ACL performed all the statistical analyses, ML, GM, RR and GL chritically reviewed the study design and its scientific contents, FV and SB reviewed the final version of the paper, AA and ADAM cohordinated the participating centres and reviewed the scientific contents of the study, ADB, ML, GM, RR, GL, FV and SB enrolled and followed patients in the participating centres. All authors have read and approved the final version of the paper.

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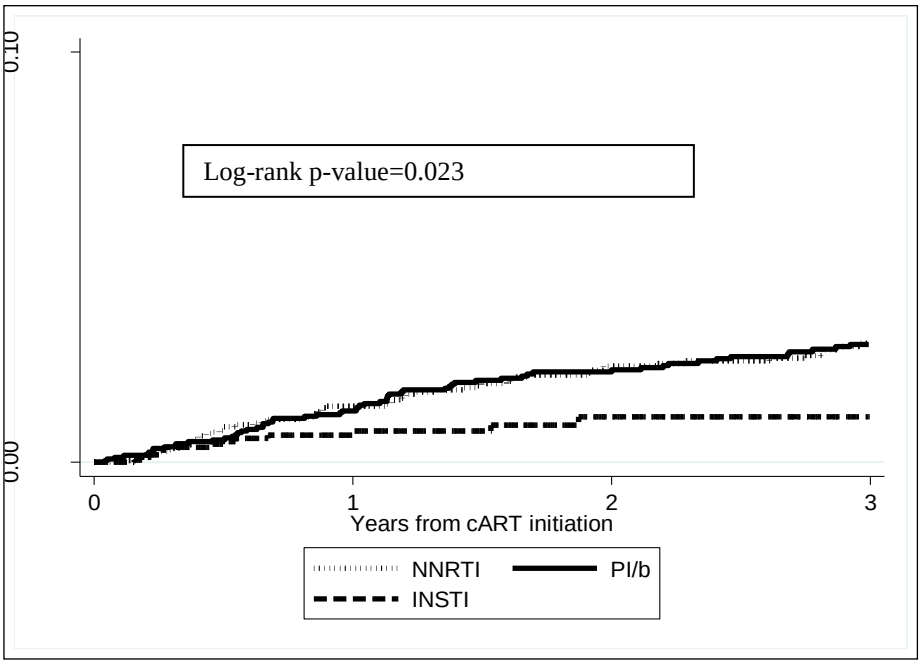
Figure 1: cumulative probability of LEE of grade ≥ 2, according to the third drug used in first-line ART, in association with 2 NNRTI, ritonavir or cobicistat-boosted PI (PI/b) or integrase strand transfert inhibitors (INSTI). The box below the figure shows 1- and 2-years probability of developing LEE (1YP and 2YP) according to ART regimen.



	1YP	95% CI	2YP	95%CI
NNRTI	1.4	1.0-2.0	2.4	1.8-3.1
PI/b	1.3	0.9-1.8	2.2	1.7-2.9
INSTI	0.7	0.4-1.2	1.1	0.6-2.0

LEE. Liver Enzime Elevations; ART: antiretroviral therapy; NNRTI:non nucleoside reverse transcriptase inhibitors; PI/b: ritonavir or cobicistat-boosted protease inhibitors; INSTI: integrase strand transfert inhibitors; 1YP: one year probability of developing LEE; 2YP: two-years probability of developing LEE.

Table 1: Main characteristics of 6575 study participants at cART initiation (baseline). If not otherwise specified, data are given as absolute numbers and percentages.

	All study population N=6575	NNRTI N=2384	PI/b N=2436	INSTI N=1755	p-value
Gender					
male	3954 (80.8%)	1995 (86.7%)	1840 (75.5%)	1449 (82.6%)	<0.001
female	941 (19.2%)	389 (16.3%)	596 (24.5%)	306 (17.4%)	
Age, years					
<35	2481 (37.7%)	963 (40.4%)	878 (36.1%)	640 (36.5%)	<0.001

35-45	2109 (32.1%)	783 (32.8%)	812 (33.3%)	514 (29.3%)	
45+	1985 (30.2%)	638 (26.8%)	746 (30.6%)	601 (34.2%)	
Mode of HIV transmission					
heterosexual	2553 (38.8%)	853 (35.8%)	1091 (44.8%)	609 (34.7%)	<0.001
IVDU	465 (7.1%)	163 (6.8%)	215 (8.8%)	87 (5.0%)	
homosexual	3045 (16.3%)	1198 (50.3%)	934 (38.3%)	913 (52.0%)	
Other/unknown	512 (7.8%)	170 (7.1%)	196 (8.1%)	146 (8.3%)	
BMI					
underweight	252 (3.8%)	69 (2.9%)	121 (5.0%)	62 (3.5%)	<0.001
normal	3214 (48.9%)	1240 (52.0%)	1202 (49.3%)	772 (44.0%)	
overweight	993 (15.1%)	407 (17.1%)	360 (14.8%)	226 (12.9%)	
obesity	225 (3.4%)	84 (3.5%)	85 (3.5%)	56 (3.2%)	
missing	1891 (28.8%)	584 (24.5%)	668 (27.4%)	639 (36.4%)	
CDC stage C	606 (9.2%)	104 (4.4%)	315 (12.9%)	187 (10.7%)	<0.001
Duration of HIV infection, years, median (IQR)	0.3 (0.1-2.3)	0.9 (0.2-3.3)	0.2 (0.1-2.0)	0.1 (0.1-0.7)	<0.001
CMV IgG					
negative	360 (5.5%)	127 (5.3%)	137 (5.6%)	96 (5.5%)	0.156
positive	2733 (41.5%)	973 (40.8%)	1058 (43.4%)	702 (40.0%)	
missing	3482 (53.0%)	1284 (53.9%)	1241 (51.0%)	957 (54.5%)	
HBsAg					
negative	5010 (76.2%)	1826 (76.6%)	1866 (76.6%)	1318 (75.1%)	0.722
positive	253 (3.9%)	87 (3.7%)	97 (4.0%)	69 (3.9%)	
missing	1312 (19.9%)	471 (19.7%)	473 (19.4%)	368 (21.0%)	
HCVAb & HCV RNA					
HCVAb -	5131 (78.0%)	1887 (79.2%)	1877 (77.1%)	1367 (77.9%)	<0.001
HCVAb+ & HCV RNA-	103 (1.6%)	40 (1.7%)	40 (1.6%)	23 (1.3%)	
HCVAb+ & HCV RNA+	381 (5.8%)	128 (5.4%)	181 (7.4%)	72 (4.1%)	
HCVAb+ & HCV RNA ND	87 (1.3%)	32 (1.3%)	34 (1.4%)	21 (1.2%)	
missing	873 (13.3%)	297 (1.6%)	304 (12.5%)	272 (15.5%)	
HCV genotype					
1	137 (2.1%)	39 (1.6%)	68 (2.8%)	30 (1.7%)	0.010
3	73 (1.1%)	26 (1.0%)	36 (1.4%)	11 (0.7%)	
4	21 (0.3%)	10 (0.4%)	6 (0.3%)	5 (0.3%)	
other	34 (0.5%)	13(0.6%)	16 (0.7%)	5 (0.3%)	
HCV Ab-	5131 (78.0%)	1887 (79.2%)	1877 (77.0%)	1367 (77.9%)	
missing	1179 (18.0%)	409 (17.2%)	433 (17.8%)	337 (19.1%)	
CD4 cell/mm³					
<200	1733 (26.4%)	308 (12.9%)	908 (37.3%)	517 (29.5%)	<0.001
201-350	1611 (24.5%)	643 (27.0%)	657 (27.0%)	311 (17.7%)	
351-500	1610 (24.5%)	785 (32.9%)	479 (19.7%)	346 (19.7%)	
500+	1362 (20.7%)	538 (22.6%)	301 (12.4%)	523 (29.8%)	
missing	259 (3.9%)	110 (4.6%)	91 (3.6%)	58 (3.3%)	
CD8 cell/mm³, median (IQR)	889 (613-1268)	947 (681-1335)	842 (561-1203)	861 (582-1271)	<0.001

HIV RNA, log_{10} copies/mL, median (IQR)	4.72 (4.14-5.22)	4.50 (4.00-4.94)	4.93 (4.31-5.42)	4.79 (4.19-5.31)	<0.001
BL ALT, median (IQR)	26 (18-40)	26 (18-38)	26 (18-41)	26 (19-40)	0.073
BL AST, median (IQR)	25 (20-33)	24 (19-32)	25 (19-35)	25 (20-34)	<0.001
GGT					
normal	4118 (62.6%)	1626 (68.2%)	1430 (58.7%)	1062 (60.5%)	<0.001
high	1787 (27.2%)	505 (21.2%)	800 (32.8%)	482 (27.5%)	
missing	670 (10.2%)	253 (10.6%)	206 (8.5%)	211 (12.0%)	
Total bilirubin					
normal	5593 (85.0%)	2047 (85.9%)	2085 (85.5%)	1461 (83.2%)	0.004
high	491 (7.5%)	176 (7.3%)	189 (7.8%)	126 (7.2%)	
missing	491 (7.5%)	161 (6.8%)	162 (6.7%)	168 (9.6%)	
Direct bilirubin					
normal	3707 (56.4%)	1510 (63.3%)	1450 (59.5%)	747 (42.5%)	<0.001
high	545 (8.3%)	185 (7.8%)	229 (9.4%)	131 (7.5%)	
missing	2323 (35.3%)	689 (58.9%)	757 (31.1%)	877 (50.0%)	
FIB-4 index					
<1.45	4782 (72.7%)	1845 (77.4%)	1686 (69.1%)	1251 (71.3%)	<0.001
145-3.25	1187 (18.1%)	361 (15.2%)	503 (20.7%)	323 (18.4%)	
>3.25	279 (4.2%)	65 (2.7%)	128 (5.3%)	86 (4.9%)	
missing	327 (5.0%)	113 (4.7%)	119 (4.9%)	95 (5.4%)	
Alchol use					
absteiners	2479 (37.7%)	937 (39.3%)	1037(42.6%)	505 (28.8%)	<0.001
occasional	1634 (24.8%)	613 (25.7%)	526 (21.6%)	495 (28.2%)	
daily	479 (7.3%)	184 (7.7%)	195 (8.0%)	100 (5.7%)	
missing	1983 (30.2%)	650 (27.3%)	678 (27.8%)	655 (37.3%)	
Current drug use					
no	5034 (76.6%)	1935 (81.2%)	1934 (79.4%)	1165 (66.4%)	<0.001
yes	172 (2.6%)	56 (2.3%)	75 (3.1%)	41 (2.3%)	
missing	1369 (20.8%)	393 (16.5%)	427 (17.5%)	549 (31.3%)	
NRTI backbone					
TDF+FTC	5391 (82.0%)	2204 (92.4%)	1998 (82.0%)	1189 (67.8%)	<0.001
other	1184 (18.0%)	180 (7.6%)	438 (18.0%)	566 (32.2%)	
Third drug					
EFV	1252 (19.0%)				
DRV/r	1120 (17.0%)				
RPV	1051 (16.0%)				
ATV/r	945 (14.4%)				
DGV	788 (12.0%)				
EVG	647 (9.8%)				
LPV	350 (5.3%)				
RGV	320 (4.9%)				
other	102 (1.6%)				

3TC: lamivudine; ABC: abacavir; ATV: atazanavir; BL: baseline; CD4: CD4+ T-lymphocites; CD8: CD8+ T-lymphocites; DRV: darunavir; DTG: dolutegravir; EFV: efavirenz; EVG: elvitegravir; FTC: emtricitabine; INSTI: integrase strand transfert inhibitors; IQR: interqualtile range; LPV: lopinavir; NNRTI: non-nucleoside reverse transcriptase inhibitors; NRTI: nucleoside reverse transcriptase inhibitors; PI/b: ritonavir or cobicistat-boosted protease inhibitors; RAL: raltegravir; RPV: rilpivirine; TDF: tenofovir; ND not determined.

Table 2: Crude (HR) and adjusted hazard ratio (AHR) for liver enzyme elevations (LEE) of grade ≥ 2 from fitting two separate multivariable model including third drug class (a) or type of third drug (b) (* time-varying covariate).

						a) model 1				b) model 2			
		HR	**95%CI**		**p**	**AHR***	**95%CI**		**p**	**AHR***	**95%CI**		**p**
Mode of HIV transmission	heterosexual	1.00				1.00				1.00			
	IVDU	3.88	2.53	5.93	0.000	0.99	0.55	1.79	0.983	0.95	0.52	1.74	0.877
	homosexual	1.41	1.00	1.98	0.050	1.30	0.90	1.87	0.165	1.30	0.90	1.87	0.164
	other/unknown	1.04	0.53	2.03	0.918	0.96	0.48	1.91	0.898	0.95	0.47	1.91	0.888
HBsAg	negative	1.00				1.00				1.00			
	positive	1.75	0.97	3.16	0.064	2.03	1.09	3.80	0.027	2.02	1.08	3.79	0.028
	missing	1.20	0.84	1.71	0.307	1.53	0.99	2.38	0.058	1.53	0.98	2.38	0.059
HCVAb & HCVRNA	HCVAb -	1.00				1.00				1.00			
	HCVAb+ & HCVRNA-	0.44	0.06	3.19	0.42	0.51	0.07	3.47	0.489	0.48	0.07	3.31	0.459
	HCVAb+ & HCVRNA+	6.26	4.50	8.72	0.000	6.29	3.94	10.05	0.000	6.36	3.96	10.21	0.000
	HCVAb+ & HCVRNA ND	2.55	0.94	6.93	0.065	3.31	1.12	9.81	0.031	3.40	1.15	10.06	0.027
	missing	0.79	0.05	1.38	0.411	0.52	0.26	1.06	0.071	0.53	0.26	1.06	0.072
Baseline CD4+ cell count	<200	1.00				1.00				1.00			
	201-350	1.08	0.73	1.61	0.698	1.02	0.61	1.71	0.942	1.02	0.61	1.71	0.950
	351-500	1.01	0.67	1.52	0.972	1.17	0.65	2.09	0.600	1.18	0.66	2.12	0.569
	500+	1.18	0.75	1.85	0.475	1.47	0.75	2.89	0.259	1.45	0.74	2.86	0.276
	missing	1.18	0.56	2.50	0.669	1.02	0.38	2.73	0.965	1.03	0.38	2.78	0.957
Baseline HIV RNA, $\log_{10}$ copies/mL	<=5	1.00				1.00				1.00			
	>5	0.84	0.61	1.15	0.272	0.78	0.54	1.12	0.180	0.77	0.53	1.12	0.173
	missing	0.99	0.50	1.96	0.982	1.02	0.41	2.54	0.973	1.01	0.41	2.54	0.975
Alcohol use at baseline	absteiners	1.00				1.00				1.00			
	occasional	1.05	0.71	1.54	0.808	1.34	0.81	2.21	0.253	1.29	0.78	2.12	0.323
	daily	1.56	0.96	2.55	0.074	1.08	0.56	2.07	0.817	1.06	0.55	2.02	0.864
	missing	0.98	0.69	1.41	0.931	0.92	0.46	1.81	0.803	1.04	0.59	1.85	0.890
Co-morbidities at baseline	no	1.00				1.00				1.00			
	yes	2.01	0.50	8.09	0.327	1.94	0.44	8.58	0.385	1.92	0.42	8.71	0.397
Concurrent medications	no	1.00				1.00				1.00			

at baseline	yes	0.93	0.64	1.36	0.725	0.91	0.54	1.53	0.723	0.93	0.55	1.56	0.775
	<200	1.00				1.00				1.00			
Current CD4+ cell count*	201-350	1.02	0.57	1.84	0.943	0.97	0.52	1.82	0.925	0.97	0.52	1.83	0.926
	351-500	0.75	0.42	1.36	0.351	0.65	0.34	1.25	0.197	0.65	0.34	1.25	0.196
	500+	0.73	0.43	1.23	0.238	0.64	0.33	1.25	0.191	0.64	0.33	1.24	0.183
	missing	0.77	0.10	5.76	0.795	0.18	0.01	2.34	0.191	0.18	0.01	2.28	0.184
Current HIV RNA	not detected	1.00				1.00				1.00			
log_{10} copies/mL*	detected <50 copies/ml	1.47	0.98	2.22	0.065	1.54	1.01	2.36	0.047	1.55	1.01	2.37	0.045
	detected >50 copies/ml	1.97	1.22	3.17	0.005	1.79	1.08	2.97	0.024	1.80	1.08	2.97	0.023
	missing	2.85	0.67	12.15	0.156	5.08	0.81	31.76	0.082	5.31	0.85	32.99	0.073
	absteiners	1.00				1.00				1.00			
Current alcohol use*	occasional	0.97	0.65	1.44	0.889	0.81	0.50	1.33	0.405	0.85	0.52	1.39	0.510
	daily	1.61	0.98	2.66	0.060	1.18	0.64	2.17	0.594	1.21	0.67	2.20	0.531
	missing	1.00	0.66	1.51	0.997	1.00	0.54	1.79	0.946	0.99	0.54	1.80	0.963
	no	1.00				1.00				1.00			
Current co-morbidities*	yes	1.21	0.52	2.82	0.660	1.15	0.48	2.73	0.760	1.15	0.48	2.76	0.749
Current non	no	1.00				1.00				1.00			
ARV- medications*	yes	1.04	0.75	1.45	0.805	1.06	0.69	1.63	0.793	1.04	0.67	1.60	0.86
NRTI backbone	Truvada vs other	1.12	0.73	1.70	0.610	0.94	0.59	1.51	0.811	0.95	0.59	1.54	0.843
	2NRTI+NNRTI	1.00				1.00							
Third drug class	2NRTI+PI/b	1.03	0.76	1.40	0.838	0.95	0.68	1.34	0.778				
	2NRTI+INSTI	0.48	0.27	0.85	0.012	0.46	0.25	0.86	0.014				
	2NRTI+NNRTI	1.00								1.00			
Third drug	2NRTI+PI/b	0.90	0.58	1.39	0.619					0.95	0.68	1.34	0.789
	DGV	0.90	0.54	1.48	0.669					0.61	0.25	1.49	0.280
	EVG	1.05	0.69	1.59	0.824					0.66	0.28	1.55	0.338
	RAL	0.51	0.22	1.19	0.120					0.11	0.02	0.84	0.033

Abbreviations: HR: hazard ratio; AHR: adjusted hazard ratio; 95%CI: 95% Confidence Interval; IVDU: intravenous drug use.

Table 3. Incidence rate, number of events, person-years of follow-up (PYFU) of grade ≥2 and ≥3 liver enzyme elevations (LEE) for third drug class stratified by HCV and HBV coinfection.

	HCVAb -					HCVAb +					p at interaction test
	Grade ≥2 LEE	PYFU	IR	95%CI		Grade ≥2 LEE	PYFU	IR	95%CI		0.117
NNRTI	53	6900	7.72	5.90	10.11	21	638	32.91	21.46	50.48	
PI/b	49	7500	6.51	4.92	8.62	33	913	36.15	25.70	50.85	
INSTI	11	2100	5.35	2.96	9.66	2	186	10.74	2.69	42.94	
	Grade ≥3 LEE	PYFU	IR	95%CI		Grade ≥3 LEE	PYFU	IR	95%CI		0.590
NNRTI	26	6900	3.74	2.55	5.50	13	662	19.63	11.40	33.81	
PI/b	20	7600	2.63	1.70	4.08	18	972	18.53	11.67	29.41	
INSTI	8	2100	3.88	1.94	7.76	1	187	5.34	0.75	37.93	
	HbsAg -					HbsAg +					p at interaction test
	Grade ≥2 LEE	PYFU	IR	95%CI		grade ≥2 LEE	PYFU	IR	95%CI		0.190
NNRTI	55	6500	8.40	6.45	10.94	6	327	18.35	8.24	40.84	
PI/b	65	7400	8.81	6.91	11.24	4	400	10.00	3.75	26.63	
INSTI	11	1900	5.67	3.14	10.23	2	108	18.43	4.61	73.68	
	grade ≥3 LEE	PYFU	IR	95%CI		grade ≥3 LEE	PYFU	IR	95%CI		0.870
NNRTI	31	6600	4.69	3.30	6.67	4	338	11.85	4.45	31.57	
PI/b	30	7500	4.01	2.81	5.74	2	405	4.94	1.24	19.76	
INSTI	7	1900	3.60	1.71	7.54	2	108	18.43	4.61	73.68	