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Original article

# Very high pre-therapy viral load is a predictor of virological rebound in HIV-1-infected patients starting a modern first-line regimen

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## Abstract

**Background: Pre-cART (combined antiretroviral therapy) plasma viral load >500,000 copies/mL has been associated with a lower probability of achieving virological suppression, while few data about its role on maintenance of virological suppression are available. In this study we aimed to clarify whether high levels of pre-cART viremia are associated with virological rebound (VR) after virological suppression.**

**Methods: HIV-infected individuals who achieved virological suppression after first-line cART were included. VR was defined as the first of two consecutive viremia >50 copies/mL (VR50) or, in an alternative analysis, >200 copies/mL (VR200). The impact of pre-cART viremia on the risk of VR was evaluated by survival analyses.**

**Results: Among 5,766 patients included, 59.2%, 31.4%, 5.2% and 4.2% had pre-cART viremia ≤100,000, 100,001-500,000, 500,001-1,000,000, and >1,000,000 copies/mL, respectively.**

**Patients with pre-cART viremia levels >1,000,000 copies/mL had the highest probability of VR (>1,000,000; 500,000-1,000,000; 100,000-500,000; <100,000 copies/mL; VR50: 28.4%; 24.3%; 17.6%; 13.8%, p<0.0001; VR200: 14.4%; 11.1%; 7.2%; 7.6%; p=0.009).**

**By Cox multivariable analyses, patients with pre-cART viremia >500,000 and >1,000,000 copies/mL showed a significantly higher risk of VR regardless of the VR endpoint used. No difference in the risk of VR was found between patients with pre-cART viremia ranging 500,000-1,000,000 copies/mL and those with pre-cART viremia >1,000,000 copies/mL, regardless of the VR endpoint used.**

**Conclusions: Pre-cART plasma viral load levels >500,000 copies/mL can identify fragile patients with poorer chance of maintaining virological control after an initial response. An effort in defining effective treatment strategies is mandatory for these patients that remain difficult to treat.**

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Running head: Pre-cART viremia & virological rebound.

## Introduction

Despite the overwhelming success of the combined antiretroviral therapy (cART) [1–4], in some patients starting their first treatment, the effectiveness of cART is still not sufficient, with consequent residual viral replication and virological failure [5–8]. Studies underlined that high pre-cART plasma viral load significantly contributes to reduce the chances of HIV-1 suppression after first-line therapy [9–12]. Initial studies indicated that subjects with plasma viral load >100,000 copies/mL at cART initiation had a reduced chance of responding to treatment [13]. However, this threshold set at 100,000 copies/mL might be not optimal to identify patients with potentially less options to achieve and maintain virological suppression (VS), particularly at the time of new and more effective antiviral approaches. In fact, recent evidences highlighted that higher levels of pre-cART viral load (such as >500,000 copies/mL) are associated with a prolonged time and a lower probability of achieving VS

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both in randomized and observational studies [9–11,14]. An association between pre-cART viral load and the risk of virological rebound (VR) after the achievement of VS was also demonstrated [9,12], though data are still limited, mainly for patients with very high pre-cART viral load levels (>500,000 or >1,000,000 copies/mL).

Patients with very high pre-cART viral load induce uncertainty in clinicians' decision because treatment recommendation for this problematic category of patients are still missing [15,16]. In this context, treatments including integrase inhibitors (INIs) or even four-drugs based strategies together with ritonavir/cobicistat boosted protease inhibitors (PIbs), seem to perform better that the classical 3-drug regimens based on PIs or non-nucleoside reverse transcriptase inhibitors (NNRTIs) [10,12].

In this analysis we evaluated the association between pre-cART viral load (with particular attention to values above 500,000 and 1,000,000 copies/mL) and the risk of VR (and potential subsequent selection of drug-resistance) in a large multi-centric cohort of HIV-1 infected patients who initially achieved VS.

# Materials and Methods

## Study population

HIV-1-infected patients were selected from: i) a large Italian anonymous database collecting data for HIV-1-infected patients followed at several clinical centres in Central Italy; ii) the Icona Foundation Study, a cohort of HIV-infected patients, which superseded the original Italian Cohort of Antiretroviral-Naive Patients study [17]. Eligible individuals were those drug-naïve who achieved VS (the first viremia <50 copies/mL after cART start, regardless of therapy changes) after starting a first-line regimen. Participants had to satisfy the additional following criteria: i) age ≥18 years; ii) first-line therapy based on at least three drugs among at least two antiretroviral classes; iii) pre-cART viremia >500 copies/mL; iv) quantifiable viremia at levels >500,000 copies/mL; vi) at least one available viral load measurement after the date of achieving VS. Patients with documented acute infection were excluded. Analyses were performed by stratifying patients in the following pre-cART strata [9–11,18]: ≤100,000, 100,001-500,000, 500,001-1,000,000, and >1,000,000 copies/mL. Reference viremia levels (dummies) were <100,000 and 500,000-1,000,000 copies/mL in order to evaluate the risks of VR in patients with pre-cART viremia ranging 100,000-500,000 and >1,000,000 copies/mL.

## Ethical approval

This study was conducted on data collected for clinical purposes. All data used in the study were previously anonymized, according to the requirements set by Italian Data Protection Code (leg. decree 196/2003) and by the General authorizations issued by the Data Protection Authority. Written informed consent for medical procedures/interventions performed for routine treatment purposes was collected for each patient included in the Icona Foundation Study or from other clinical centers involved in the study, in accordance with the ethics standards of the committee on human experimentation and the Helsinki Declaration (1983 revision).

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## Statistical analysis

All the analyses were performed using R open source environment for statistical computing (version 3.4.1).

## Survival analysis: virological rebound

Survival analysis was used to estimate the cumulative probability and predictors of experiencing VR. The date of VR was defined according the two following definitions: i) the date of the first of two consecutive viral load measurements >50 copies/mL (VR50); ii) the date of the first of two consecutive viral load measurements >200 copies/mL (VR200). Survival analyses were performed by ignoring therapy changes and patients' follow-up was censored at the date of their last available viremia measurement or at the time of the first therapy interruption (intention to treat approach, ITT). Kaplan-Meier curves were performed to estimate the probability of VR according to pre-cART viremia strata. Cox regression analysis was performed to evaluate the association of pre-cART viral load on risk VR after controlling for other potential confounding factors, such transgender, age, HIV-1 subtype, mode of HIV-1 transmission, year of cART initiation (per 1 increase of calendar year), pre-cART viral load (categorised as indicated above), pre-cART CD4 cell count (per 100 cells/$mm^3$ increase), type of initial regimen started, type of NRTI-backbone used, time (months) to achieving VS (categorised as: <6 months; 6-12 months; >12 months), and level of transmitted drug resistance detected at pre-cART genotypic resistance test (GRT). Cox regression models were performed under the assumption of proportionality of the hazards. To avoid any potential bias due to missing data, for variables containing missing values (HIV-1 subtype; risk factor; transmitted drug resistance, calculated according to the list of Bennet *et al.* [19]) a Multiple Imputation statistical approach was performed by using MICE (Multivariate Imputation via Chained Equations) package of R. Moreover, a model excluding variables with missing values was also built for confirmation.

## Evaluation of resistance detected after virological rebound

Among patients with an available plasma GRT performed within 6 months after VR, the prevalence of primary resistance mutations (PRMs, according to the list panelled on Stanford HIV Drug Resistance Database [https://hivdb.stanford.ed]) was evaluated and compared according to the levels of viremia at GRT by using Chi-squared test for trend.

## Sensitivity analyses

To confirm the robustness of the results, the following additional Cox regression models were performed: i) excluding patients who started a rilpivirine-containing regimen because the usage of this NNRTI is recommended only in patients with pre-cART viremia <100,000 copies/mL [15,16]; ii) by censoring patients at the end of their first-line regimen (on treatment approach, OT).

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# Results

## Patients' characteristics

Overall, 5,766 patients were included in the study. Patients' characteristics are summarized in Table 1. Patients started their first-line regimen on average in 2011 (Median [first quartile-third quartile: $Q_1$-$Q_3$] 2011 [2008-2013]). Stratifying patients according to viremia ranks, 59.2%, 31.4%, 5.2% and 4.2% had their pre-cART viremia value in the ranges of ≤100,000, 100,001-500,000, 500,001-1,000,000, and >1,000,000 copies/mL, respectively.

The proportion of patients who achieved VS before 6 months, over 6-12 months and after 12 months of the date of treatment initiation was 67.7%, 24.1% and 9.2%, respectively. Regarding first-line treatments, the most commonly used PIb was lopinavir (911, 34.1%), followed by atazanavir (861, 32.2%) and darunavir (780, 29.2%). Efavirenz (1,771, 71.7%) was the most commonly administered NNRTI, followed by rilpivirine (506, 20.5%) and nevirapine (189, 7.7%). Finally, raltegravir was the most commonly used INI (262, 49.6%), followed by elvitegravir (165, 31.3%) and dolutegravir (101, 19.1%). Patients with higher pre-cART viremia levels were more likely to have started a triple therapy containing a PIb, an INI or a therapy containing at least four drugs, while people with lower pre-cART viremia levels were mainly treated with an NNRTI-based triple therapy ($p<0.001$; Table 1).

Transmitted drug resistance was observed in the 9.6% (PI [0.8%]; NRTI [3.3%]; NNRTI [5.9%]) of patients with an available GRT at cART baseline (N=3,038).

# Survival analyses: virological rebound

## i) Virological rebound: 50 copies/mL threshold (VR50)

Overall, by four years from the date of achieving VS, the risk of experiencing VR50 was 16.2% (95% confidence interval, CI: 15%-17.3%). Median (IQR) viral load at VR50 was 208 (87-5,834) copies/mL. Patients who experienced VR50 were under virological suppression since a median (IQR) time of 12.4 (4.3-30.8) months.

Stratifying patients by pre-cART viremia ranges, those with pre-cART viremia levels >1,000,000 copies/mL had the highest probability of VR50 and a dose-response relationship of decreasing risks with lower viremia level was observed (>1,000,000: 28.4%; 500,001-1,000,000: 24.3%; 100,001-500,000: 17.6%; ≤100,000: 13.8%; $p<0.0001$; Figure 1, panel A).

Cox multivariable regression model confirmed the impact of pre-cART viremia onVR50. In fact, the adjusted hazard ratio (aHR) of experiencing VR50 was significantly higher in patients having pre-cART viremia levels >1,000,000 copies/mL and 500,001-1,000,000 copies/mL, compared to those having pre-cART viremia ≤100,000 copies/mL ($p<0.0001$; Table 2).

To evaluate potential differences in terms of VR risk between pre-cART viremia strata 500,000-1,000,000 and >1,000,000 copies/mL, we repeated the same Cox regression models by

considering as reference category for pre-cART viremia the stratum 500,000-1,000,000 copies/mL (Table 2). We found no evidence that the risk of VR50 in patients with pre-cART viremia >1,000,000 was different to that of those with pre-cART viremia ranging 500,000-1,000,000 copies/mL (aHR [95% CI]: 1.03 [0.72-1.49], p=0.860). Whereas, compared to those belonging 500,000-1,000,000 copies/mL stratum, patients with pre-cART viremia <100,000 and 100,000-500,000 copies/mL had a significant lower aHR of VR (Supplementary Table 1).

Other factors associated with a higher risk of VR50, independently of pre-cART viremia, were to be drug abuser versus to be homosexual and carrying a non-B subtype HIV-1 versus the more common B subtype (Table 2). In contrast, factors associated with a lower aHR of experiencing VR50 were a more recent year of first-line cART initiation, a first-line regimen based on two NRTIs plus one NNRTI (compared to PI-based regimens), and higher levels of pre-cART CD4 cell count.

## ii) Virological rebound: 200 copies/mL threshold (VR200)

Overall, by four years from the date of achieving VS, the risk of experiencing VR200 was 7.9% (95% CI: 7.0%-8.7%). Median (IQR) viral load at VR200 was 6,938 (742-53,649) copies/mL. Patients who experienced VR200 were under virological suppression since a median (IQR) time of 16.7 (7.3-39.0) months.

Again, patients with pre-cART viremia levels >1,000,000 and 500,001-1,000,000 copies/mL had the highest probability of VR200 (>1,000,000 copies/mL: 14.4%; 500,001-1,000,000 copies/mL: 11.1%; 100,001-500,000 copies/mL: 7.2%; ≤100,000 copies/mL: 7.6%; p=0.009; Figure 1, panel B). Cox multivariable regression model showed results similar to those observed when using the >50 copies/mL threshold endpoint (Table 2).

Also for the endpoint VR200, we found no evidence that the risk of VR in patients with pre-cART viremia >1,000,000 was different to that of those with pre-cART viremia ranging 500,000-1,000,000 copies/mL (aHR [95% CI]: 1.02 [0.60-1.75], p=0.942; Supplementary table 1).

## Sensitivity analyses

Considering that rilpivirine has been approved only for treating patients with pre-cART viremia <100,000 copies/mL [15,16], to avoid potential bias related with pre-cART viremia due to this recommendation, we performed a Cox regression model after excluding patients who started ART with rilpivirine. Results of the model were superimposable with those obtained from the full set and the VR50 endpoint (Supplementary Table 2).

We repeated Cox regression analyses by censoring patients' follow-up at the date of their last viremia measurement available during the first-line treatment (OT approach); results were similar to those obtained from ITT approach (Supplementary table 3).

Finally, we repeated Cox regression analyses by excluding variables with missing values (HIV-1 subtype, risk factor and transmitted drug resistance). The final model showed results superimposable to the those observed in the model built by using MICE approach (data not shown).

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### Overview of resistance detected after VR

Among 709 patients experiencing VR, 170 (24.0%) had an available GRT after VR; 71 (41.8%) of them had at least one PRM. In particular, 9 (5.3%), 48 (28.2%) and 49 (28.8%) patients had at least one PI, NRTI and NNRTI PRM, respectively. The proportion of patients with resistance to any drug class was significantly higher with higher viremia levels at the time of GRT after VR (ranging from 27.0% at GRT viremia of 51-200 copies/mL to 36.4% at viremia >100,000 copies/mL, with a peak of 61.5% at viremia of 1,000-10,000 copies/mL, P=0.007, Figure 2).

We also evaluated the emergence of resistance after VR according to pre-cART viremia levels. By cross tabulating the detection of PRMs with pre-cART viremia levels, a considerable proportion of patients with resistance after VR was also found in those with very high pre-cART viremia (pre-cART viremia [copies/mL]: % of patients with ≥1 PRM after VR: <100,000: 39.6%; 100,001-500,000: 48.6%; 500,001-1,000,000: 27.3%; >1,000,000: 44.4%, p=0.854).

Sixty-seven out of 170 patients had the GRT available after VR under the first-line treatment without any therapy switch. Among them, the proportion of patients with at least one PRM after VR was lower among subjects receiving a PI-based cART (12/37, 32.4%) compared to those receiving a NNRTI-based cART (17/30, 56.7%, p=0.046).

Regarding INI resistance, integrase GRT after VR was available for 31 patients. Among these, three patients (9.7%) developed the INI PRM N155H.

We also performed an analysis on a sub-group of patients who had both a GRT before cART start and a GRT after VR (114/170, 67.1%), to evaluate the extent of newly PRM selected. Among the 40 (35.1%) patients with resistance after VR, almost all (38, 95%) developed new PRMs.

## Discussion

In the present manuscript, by analysing a large cohort of HIV-1 infected patients followed in several clinical centres in Italy who initially achieved VS, we observed a strong association between pre-cART viral load and the risk of VR after VS (by both ITT and OT approaches, also after adjusting for several confounding factors), confirming the negative role of very high viremia levels (>500,000 copies/mL) on the maintenance of virological control [9]. While in previous analysis we compared patients with a viral load below or above 500,000 copies/mL [9], here, thanks to the much larger sample size, we could explore the issue more in depth by using finer viremia categories, including values >1,000,000 copies/mL. We observed a significantly raised risk of VR in patients who started with pre-cART viremia levels of 500,001-1,000,000 copies/mL as compared to those who started with a value <100,000 copies/mL. No further increase in risk was significantly present for patients starting with viremia values >1,000,000 copies/mL. Thus, our results indicate that very high pre-cART viremia (>500,000 copies/mL) seems to be more strongly associated with a negative response to first-line therapy, compared to the widely used levels of 100,000 copies/mL.

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Raffi *et al.* in a recent publication also showed that pre-cART viral load is negatively associated with the chance of maintaining virological control after first-line therapy initiation [12]. However, the authors found a difference in risk of VR when comparing pre-cART viremia levels > or <100,000 copies/mL but no further differences in the strata of 100,000-500,000 and >500,000 copies/mL [12]. This discrepancy might be explained by the fact that only patients with treatments including two NRTIs plus one PI or one INI or efavirenz were included in this French study. Indeed, selection might underestimate the number of patients with very high pre-cART viral load that might receive alternative treatments [10]. For this reason, in our analysis, we decided to include all patients treated with at least three drugs among at least two antiretroviral classes (regardless the recommended first-line regimens) to better represent real settings.

Of note, we found that NNRTI-based therapies (regardless of rilpivirine use) were associated with a significant lower risk of VR than PIb-based therapies. However, we found that the usage of NNRTIs is associated with a higher rate of resistance selection after VR compared to PIs, confirming that NNRTIs, despite their high potency, have a lower genetic barrier to develop resistance compared to PIs.

Regarding virological response under INIs, also this drug-class performed better than PIs, even though only at univariable analyses (probably due to the very low number of rebounds documented under INI-based treatments; Table 2). These results regarding the drug-class comparison are consistent with data already available [10,12,20,21].

In our study we also explored the prevalence of resistance after VR according to viremia levels at GRT. We detected a considerable rate of resistance also at low-level viremia (27% at viremia 51-200 copies/mL), in line with other previous studies [22–25], confirming that the presence of resistance at these low viremia ranges is not a rare event. In this context, considering that GRT is reliable even at low-level viremia [22–25], resistance in patients experiencing rebound, especially with high pre-cART plasma viral load, should be promptly tested after rebound regardless viremia magnitude to avoid virological failure and/or loss of treatment options related to resistance development. Indeed, resistance detected at low-level viremia has been already associated with an increased risk of virological failure [26].

Our analysis has a number of limitations. First, important potential non-measured confounders such as adherence levels and information about acute/recent seroconversion were not evaluated because poorly recorded in our database. Concerning adherence, even though patients might have a good initial compliance because the majority of them achieved undetectability under their first drug regimen, we found that drug abuser patients had an increased risk of experiencing VR. These results might reflect an indirect association between low adherence and VR, as recently observed in study conducted on Swiss Cohort that confirmed the association of drug abuse with poorer adherence and consequently with virological failure [27]. By contrast, we found that patients receiving an NNRTI-based regimen had a lower of risk of experiencing VR compared to those treated with a PI-based regimen. This finding may reflect clinician preference for prescribing PI-based cART to patients perceived to be at risk for poor adherence [28]. Another point is that we cannot extrapolate

robust results from patients starting INI-based treatment. Due to the extraordinary INIs efficacy, we observed very few rebound events. Further studies including a larger number of patients treated with INIs are required to provide more robust results regarding this drug class.

In conclusion, pre-cART viremia >500,000 copies/mL is a condition that can identify patients with lower chances of maintain virological control after initial undetectability. An effort in defining effective treatment strategies is mandatory for these patients that remain difficult to treat.

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## Legends to figures

Figure 1. Kaplan-Meier curves estimates of cumulative probability of virological rebound according to pre-therapy plasma viral load ranges by four years.

Kaplan-Meier cumulative probability estimates of VR (the first of two consecutive plasma viral load measurements >50 copies/mL, Panel A; the first of two consecutive plasma viral load measurements >200 copies/mL, Panel B) at four years after achieving virological suppression was performed by stratifying patients according to pre-therapy viral load ranges (copies/mL). Analyses were performed regardless therapy changes and patients were censored at the last viremia measurement available or at the time of the first therapy interruption. P-values were calculated by using log-rank test for trend. A p-value <0.05 was considered statistically significant. VR: virological rebound. VS: virological suppression.

Figure 2. Resistance prevalence detected after VR according to pre-therapy viral load ranges.

Line plots represent the proportion of patients with resistance detected after VR considering resistance to any drug (circle with dotted line), PI resistance (circle with continue line), NRTI resistance (triangle with dotted line) and NNRTI resistance (rhombus with dotted line). Resistance was stratified according to pre-therapy viral load ranges. P-values were calculated by using Chi Squared test for trend. A p-value <0.05 was considered statistically significant. GRT: genotypic resistance test; VR: virological rebound.

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Table 1. Characteristics of 5,766 drug naive HIV-1 infected patients achieving virological suppression after the first-line therapy stratified for pre-cART plasma viral load.

| Characteristics | Overall N=5,766 | Pre-cART plasma viral load (copies/mL) | | | | p-value[a] |
|---|---|---|---|---|---|---|
| | | ≤100,000 N=3,407 | 100,001-500,000 N=1,814 | 500,001-1,000,000 N=302 | >1,000,000 N=243 | |
| **Male, n (%)** | 4,499 (78) | **2,591 (76.0)** | **1,492 (82.2)** | **234 (77.5)** | **182 (74.9)** | **0.039** |
| **Age (years), median ($Q_1$-$Q_3$)** | 39 (33-46) | **38 (32-46)** | **40 (33-47)** | **42 (34-49)** | **42 (34-50)** | **<0.0001↑** |
| **Pre-cART CD4 cell count (cells/mm$^3$), median ($Q_1$-$Q_3$)** | 280 (153-390) | **313 (217-422)** | **238 (106-357)** | **135 (44-290)** | **99 (37-216)** | **<0.0001↓** |
| **Time (months) of achieving VS, n (%):** | | | | | | |
| *<6* | 3,846 (66.7) | **2590 (76)** | **1054 (58.1)** | **123 (40.7)** | **79 (32.5)** | **<0.0001** |
| *6-12* | 1,389 (24.1) | **593 (17.4)** | **573 (31.6)** | **128 (42.4)** | **95 (39.1)** | **<0.0001** |
| *>12* | 531 (9.2) | **224 (6.6)** | **187 (10.3)** | **51 (16.9)** | **69 (28.4)** | **<0.0001** |
| **Risk factor, n (%):** | | | | | | |
| *Homosexual* | 2,398 (41.6) | **1,369 (40.2)** | **783 (43.2)** | **145 (48)** | **101 (41.6)** | **0.021** |
| *Heterosexual* | 2,398 (41.6) | 1446 (42.4) | 738 (40.7) | 116 (38.4) | 98 (40.3) | 0.124 |
| *Drug abuser* | 512 (8.9) | 310 (9.1) | 159 (8.8) | 22 (7.3) | 21 (8.6) | 0.424 |
| *Other* | 269 (4.7) | 160 (4.7) | 82 (4.5) | 13 (4.3) | 14 (5.8) | 0.799 |
| *Unknown* | 189 (3.3) | 122 (3.6) | 52 (2.9) | 6 (2.0) | 9 (3.7) | 0.248 |
| **Transmitted drug resistance, n (%)**[b,c] | 292 (9.6) | 173 (9.8) | 83 (8.5) | 24 (15.1) | 12 (9.2) | 0.702 |
| **Subtype, n (%):** | | | | | | |
| *B* | 2,553 (44.3) | 1,504 (44.1) | 840 (46.3) | 122 (40.4) | 87 (35.8) | 0.099 |
| *CRF02_AG* | 217 (3.8) | **118 (3.5)** | **70 (3.9)** | **11 (3.6)** | **18 (7.4)** | **0.014** |
| *F* | 168 (2.9) | **77 (2.3)** | **64 (3.5)** | **13 (4.3)** | **14 (5.8)** | **<0.0001** |
| *C* | 142 (2.5) | 92 (2.7) | 31 (1.7) | 9 (3.0) | 10 (4.1) | 0.870 |
| *Other* | 358 (6.2) | **202 (5.9)** | **107 (5.9)** | **26 (8.6)** | **23 (9.5)** | **0.022** |
| *Unknown* | 2328 (40.4) | 1,414 (41.5) | 702 (38.7) | 121 (40.1) | 91 (37.4) | 0.065 |
| **First-line therapy, n (%):** | | | | | | |
| *2 NRTIs + 1 PIb* | 2,675 (46.4) | **1403 (41.2)** | **952 (52.5)** | **175 (57.9)** | **145 (59.7)** | **<0.0001** |
| *2 NRTIs + 1 NNRTI* | 2,469 (42.8) | **1668 (49.0)** | **673 (37.1)** | **84 (27.8)** | **44 (18.1)** | **<0.0001** |
| *2 NRTIs + 1 INI* | 401 (7.0) | **257 (7.5)** | **114 (6.3)** | **19 (6.3)** | **11 (4.5)** | **0.024** |
| *PIb + INI + ≥1 NRTI* | 127 (2.2) | **33 (1.0)** | **37 (2.0)** | **20 (6.6)** | **37 (15.2)** | **<0.0001** |
| *Other* | 94 (1.6) | 46 (1.4) | 38 (2.1) | 4 (1.3) | 6 (2.5) | 0.090 |
| **NRTI-backbone[d], n (%):** | | | | | | |
| *TDF/TAF+FTC* | 4,282 (74.4) | **2516 (73.9)** | **1317 (72.8)** | **250 (83.1)** | **199 (82.6)** | **0.001** |
| *ABC+3TC* | 451 (7.8) | **306 (9)** | **113 (6.2)** | **18 (6)** | **14 (5.8)** | **0.001** |
| *AZT+3TC* | 613 (10.6) | 349 (10.3) | 219 (12.1) | 24 (8.0) | 21 (8.7) | 0.832 |
| *Other* | 410 (7.2) | 233 (6.8) | 161 (8.9) | 9 (3.0) | 7 (2.9) | 0.124 |

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| | | | | | | |
|---|---|---|---|---|---|---|
| **More than three drugs, n (%)** | 219 (3.8) | **82 (2.4)** | **75 (4.1)** | **22 (7.3)** | **40 (16.5)** | **<0.0001** |
| **Year of cART initiation, median ($Q_1$-$Q_3$)** | 2011 (2008-2013) | **2011 (2008-2013)** | **2010 (2007-2012)** | **2011 (2009-2013)** | **2012 (2010-2013)** | **<0.0001↑** |
| **Plasma viral load follow-up length (years), median ($Q_1$-$Q_3$)** | 3 (1-6) | **3 (1-6)** | **3 (2-6)** | **3 (2-5)** | **3 (1-4)** | **0.010↑** |
| **No. of plasma viral load measurements per year, median ($Q_1$-$Q_3$)** | 3 (2-4) | 3 (2-4) | 3 (2-4) | 3 (2-4) | 3 (2-4) | 0.509 |

[a] P-value was calculated by Chi Squared test for trend for qualitative variables and by Jonckheere-Terpstra test (↑, alternative one-side hypothesis: increasing. ↓, alternative one-side hypothesis: decreasing) for quantitative variables. [b] Only for patients with available genotypic resistance test at baseline: N=3,038 (≤100,000 N=1,772. 100,001-500,000 N=976. 500,001-1,000,000 N=159. >1,000,000 N=131). [c] As the presence of at least one mutation from WHO surveillance transmitted resistance list (Bennet et al, 2009 [20]). [d] Only for patients under a NRTI containing regimen: N=5,756 (≤100,000 N=3,404. 100,001-500,000 N=1,810. 500,001-1,000,000 N=301. >1,000,000 N=241). cART: combined antiretroviral therapy. INI: integrase inhibitor. MVC: maraviroc. NNRTI: non-nucleoside reverse transcriptase inhibitor. NRTIs: nucleos(t)ide reverse transcriptase inhibitors. PIbs: ritonavir/cobicistat protease inhibitors. $Q_1$: first quartile. $Q_3$: third quartile. T20: enfuvirtide. VS: virological suppression. WHO: World Health Organization. Boldface indicates factors significantly associated with pre-therapy plasma viral load ranges ($p<0.05$).

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Table 2. Factors associated with virological rebound in HIV-1 infected patients achieving virological suppression after the first-line therapy.

| Variables | Hazard ratio of experiencing virological rebound (first of two consecutive plasma viral laod >50 copies/mL) | | | | Hazard ratio of experiencing virological rebound (first of two consecutive plasma viral load >200 copies/mL) | | | |
|---|---|---|---|---|---|---|---|---|
| | Crude | | Adjusted[a] | | Crude | | Adjusted[a] | |
| | HR (95% CI) | p-value | HR (95% CI) | p-value | HR (95% CI) | p-value | HR (95% CI) | p-value |
| **Gender (*female* vs. *male*[b])** | **1.30 (1.12-1.52)** | **0.001** | 1.08 (0.92-1.27) | 0.346 | **1.65 (1.34-2.04)** | **<0.0001** | **1.27 (1.02-1.58)** | **0.034** |
| **Age (per 5 years increase)** | 1.02 (0.98-1.05) | 0.338 | 1.00 (0.97-1.04) | 0.831 | 0.96 (0.92-1.01) | 0.126 | 0.96 (0.91-1.02) | 0.170 |
| **HIV-1 subtype[c] (*non-B* vs. *B*[b])** | **1.36 (1.17-1.59)** | **<0.0001** | **1.46 (1.24-1.72)** | **<0.0001** | **1.45 (1.17-1.80)** | **0.001** | **1.60 (1.27-2.01)** | **<0.0001** |
| **Mode of HIV-1 transmission[c]:** | | | | | | | | |
| *Homosexual[b]* | 1 | | 1 | | 1 | | 1 | |
| *Heterosexual* | 1.09 (0.94-1.27) | 0.270 | 1.13 (0.97-1.32) | 0.116 | 1.21 (0.96-1.51) | 0.100 | 1.23 (0.98-1.54) | 0.072 |
| *Drug abuser* | **1.79 (1.45-2.21)** | **<0.0001** | **1.49 (1.20-1.85)** | **<0.0001** | **2.60 (1.97-3.41)** | **<0.0001** | **2.08 (1.57-2.75)** | **<0.0001** |
| *Other* | 1.05 (0.74-1.51) | 0.775 | 0.98 (0.68-1.41) | 0.918 | 0.94 (0.53-1.65) | 0.822 | 0.87 (0.49-1.55) | 0.645 |
| **Year of cART initiation (per 1 year increase)** | **0.89 (0.87-0.91)** | **<0.0001** | **0.89 (0.87-0.92)** | **<0.0001** | **0.86 (0.83-0.88)** | **<0.0001** | **0.88 (0.84-0.92)** | **<0.0001** |
| **Pre-cART viral load (copies/mL):** | | | | | | | | |
| *≤100,000[b]* | 1 | | 1 | | 1 | | 1 | |
| *100,001-500,000* | **1.29 (1.11-1.50)** | **0.001** | 1.16 (0.99-1.36) | 0.068 | 0.92 (0.74-1.15) | 0.476 | 0.90 (0.72-1.13) | 0.376 |
| *500,001-1,000,000* | **2.04 (1.57-2.65)** | **<0.0001** | **1.92 (1.46-2.54)** | **<0.0001** | **1.59 (1.08-2.32)** | **0.018** | **1.85 (1.24-2.76)** | **0.003** |
| *>1,000,000* | **2.32 (1.75-3.08)** | **<0.0001** | **1.99 (1.46-2.71)** | **<0.0001** | **1.84 (1.23-2.77)** | **0.003** | **1.89 (1.21-2.95)** | **0.005** |
| **Pre-cART CD4 cell count (per 100 cells/mm$^3$ increase)[c]:** | **0.85 (0.81-0.88)** | **<0.0001** | **0.94 (0.90-0.99)** | **0.010** | **0.89 (0.83-0.94)** | **<0.0001** | 0.97 (0.91-1.03) | 0.366 |
| **Type of initial regimen started:** | | | | | | | | |
| *2 NRTIs + 1 PIb[b]* | 1 | | 1 | | 1 | | 1 | |
| *2 NRTIs + 1 NNRTI* | **0.67 (0.58-0.78)** | **<0.0001** | **0.68 (0.59-0.80)** | **<0.0001** | 0.85 (0.70-1.04) | 0.120 | **0.79 (0.64-0.98)** | **0.030** |
| *2 NRTI + INI* | **0.46 (0.29-0.74)** | **0.001** | 0.87 (0.54-1.42) | 0.586 | **0.26 (0.10-0.70)** | **0.008** | 0.52 (0.19-1.41) | 0.198 |
| *PIb + INI + ≥1NRTI* | 0.65 (0.35-1.23) | 0.186 | 0.69 (0.34-1.40) | 0.301 | 0.63 (0.24-1.71) | 0.366 | 0.70 (0.22-2.23) | 0.546 |
| *Other* | 1.22 (0.79-1.87) | 0.371 | 0.90 (0.57-1.41) | 0.634 | 1.31 (0.73-2.35) | 0.359 | 0.82 (0.45-1.52) | 0.534 |
| **Type of NRTI-backbone used:** | | | | | | | | |
| *TDF + FTC[b]* | 1 | | 1 | | 1 | | 1 | |
| *ABC + 3TC* | 0.97 (0.72-1.31) | 0.841 | 0.88 (0.65-1.19) | 0.414 | 1.19 (0.78-1.81) | 0.425 | 1.08 (0.70-1.65) | 0.739 |

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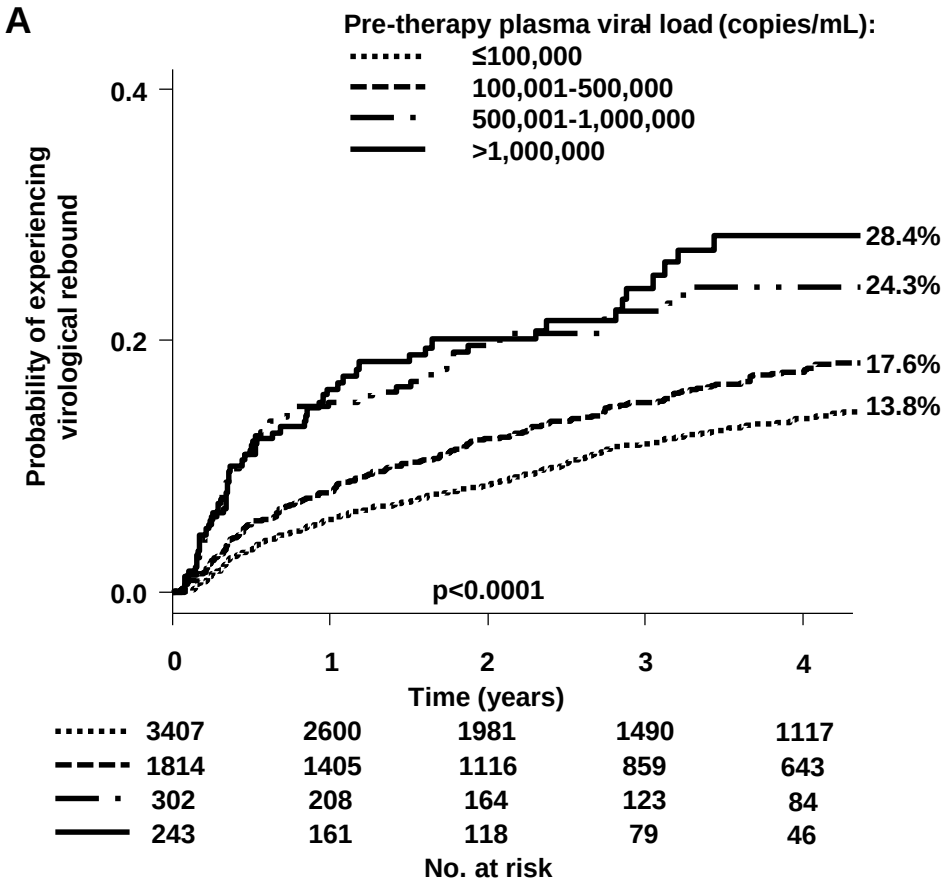
| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *AZT + 3TC* | **2.14 (1.79-2.56)** | **<0.0001** | 1.08 (0.85-1.37) | 0.529 | **2.65 (2.08-3.39)** | **<0.0001** | 1.25 (0.89-1.74) | 0.200 |
| *Other* | **1.95 (1.58-2.40)** | **<0.0001** | 1.01 (0.77-1.34) | 0.928 | **2.87 (2.20-3.76)** | **<0.0001** | 1.32 (0.91-1.91) | 0.142 |
| **Time (months) to achieving VS:** | | | | | | | | |
| *<6*[b] | 1 | | 1 | | 1 | | 1 | |
| *6-12* | 1.13 (0.95-1.33) | 0.163 | 0.98 (0.83-1.17) | 0.857 | 0.88 (0.69-1.14) | 0.336 | 0.85 (0.65-1.09) | 0.204 |
| *>12* | **1.69 (1.38-2.08)** | **<0.0001** | 1.02 (0.81-1.27) | 0.880 | **1.63 (1.22-2.17)** | **0.001** | 0.93 (0.68-1.26) | 0.631 |
| **TDR detected at pre-cART GRT**[c, d] | 0.84 (0.66-1.05) | 0.127 | 0.85 (0.67-1.07) | 0.159 | 0.80 (0.57-1.11) | 0.184 | 0.86 (0.61-1.20) | 0.379 |

[a] Adjusted for: gender, age, HIV-1 subtype, mode of HIV-1 transmission, year of cART initiation, pre-cART viral load, pre-cART CD4 cell count, type of initial regimen started, type of NRTI-backbone used, time to achieving VS and level of TDR detected at pre-cART GRT. [b] Reference group (dummy). [c] A multiple imputation approach was performed to fill missing values. [d] As the presence of at least one mutation from WHO surveillance TDR list (Bennet et al, 2009 [20]). 3TC: lamivudine. ABC: abacavir. AZT: zidovoudine. CI: confidence interval. cART: combined antiretroviral therapy. FTC: emtricitabine. GRT: genotypic resistance test. HR: hazard ratio. INI: integrase inhibitor. NNRTI: nucleoside reverse transcriptase inhibitor. NRTIs: nucleos(t)ide reverse transcriptase inhibitors. PIb: ritonavir-cobicistat boosted protease inhibitor. TDF: tenofovir. TDR: transmitted drug resistance. VS: virological suppression. WHO: world health organization. Boldface indicates factors that were significantly associated ($p<0.05$) with virological rebound.

**Figure 1**


A
Pre-therapy plasma viral load (copies/mL):
≤100,000
100,001-500,000
500,001-1,000,000
>1,000,000
Probability of experiencing virological rebound
0.4
0.2
0.0
28.4%
24.3%
17.6%
13.8%
p<0.0001
0
1
2
3
4
Time (years)
3407 2600 1981 1490 1117
1814 1405 1116 859 643
302 208 164 123 84
243 161 118 79 46
No. at risk

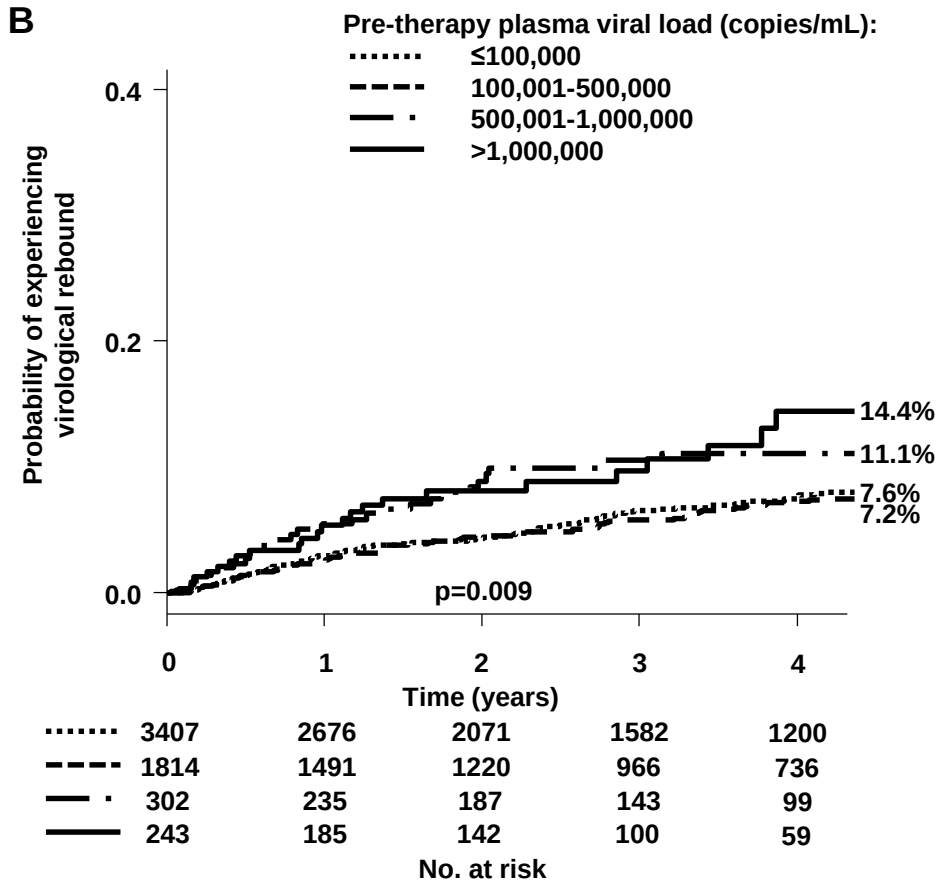
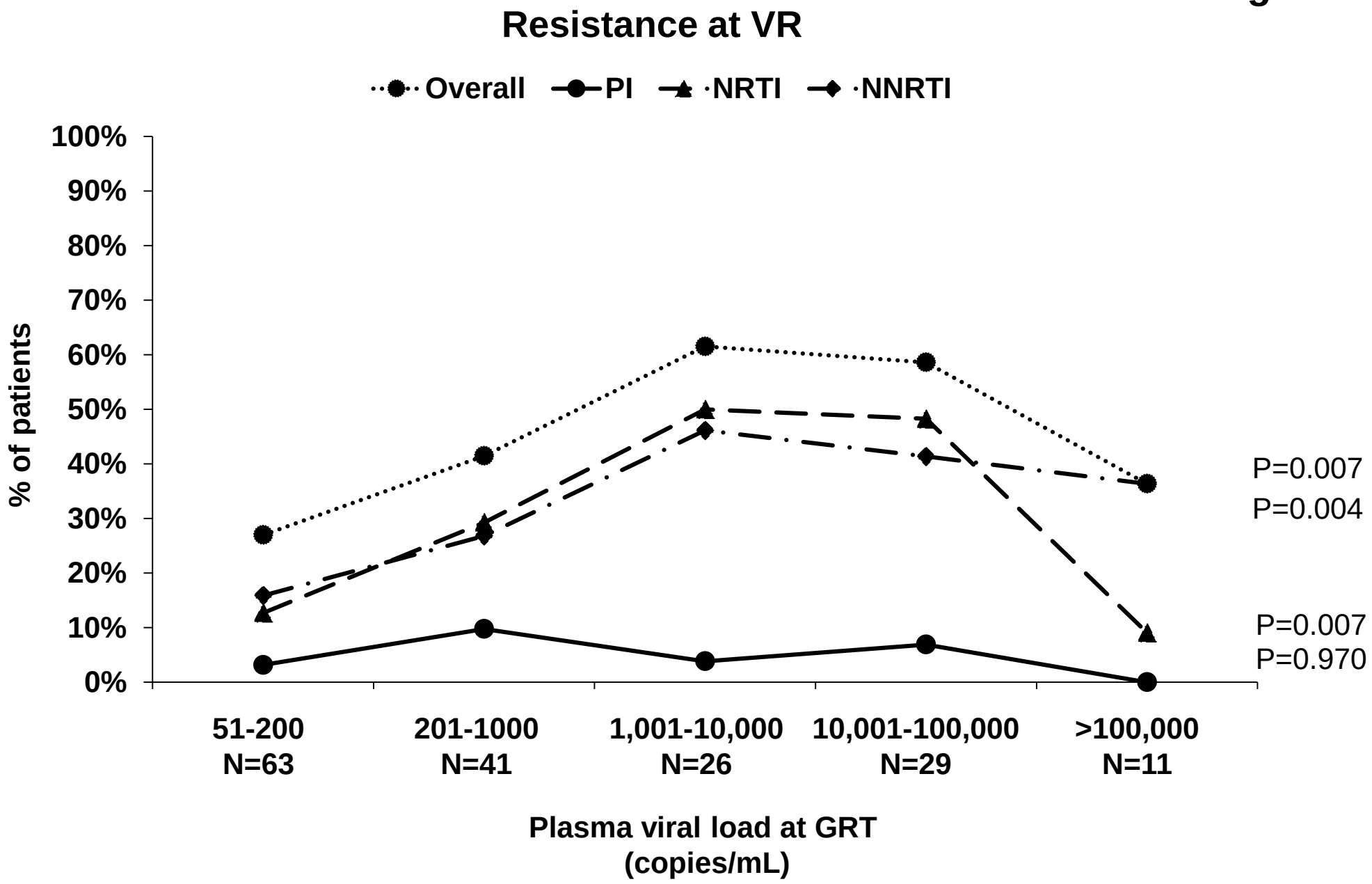

B
Pre-therapy plasma viral load (copies/mL):
≤100,000
100,001-500,000
500,001-1,000,000
>1,000,000
Probability of experiencing virological rebound
0.4
0.2
0.0
14.4%
11.1%
7.6%
7.2%
p=0.009
0
1
2
3
4
Time (years)
3407 2676 2071 1582 1200
1814 1491 1220 966 736
302 235 187 143 99
243 185 142 100 59
No. at risk

**Figure 2**


Resistance at VR
Overall
PI
NRTI
NNRTI
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
% of patients
P=0.007
P=0.004
P=0.007
P=0.970
51-200
N=63
201-1000
N=41
1,001-10,000
N=26
10,001-100,000
N=29
>100,000
N=11
Plasma viral load at GRT
(copies/mL)

**Supplementary Table 1. Factors associated with virological rebound in HIV-1 infected patients achieving virological suppression after the first-line therapy starting.**

| Variables | Hazard ratio of experiencing virological rebound (first of two consecutive plasma viral load >50 copies/mL) | | | | Hazard ratio of experiencing virological rebound (first of two consecutive plasma viral load >200 copies/mL) | | | |
|---|---|---|---|---|---|---|---|---|
| | Crude | | Adjusted[a] | | Crude | | Adjusted[a] | |
| | HR (95% CI) | p-value | HR (95% CI) | p-value | HR (95% CI) | p-value | HR (95% CI) | p-value |
| **Pre-cART viral load (copies/mL):** | | | | | | | | |
| *≤100,000* | **0.49 (0.38-0.64)** | **<0.0001** | **0.52 (0.39-0.69)** | **<0.0001** | **0.63 (0.43-0.92)** | **0.018** | **0.54 (0.36-0.81)** | **0.003** |
| *100,001-500,000* | **0.63 (0.48-0.83)** | **0.001** | **0.60 (0.46-0.80)** | **<0.0001** | **0.58 (0.39-0.87)** | **0.008** | **0.49 (0.32-0.74)** | **0.001** |
| *500,001-1,000,000*[b] | 1 | | 1 | | 1 | | 1 | |
| *>1,000,000* | 1.14 (0.79-1.63) | 0.482 | 1.03 (0.72-1.49) | 0.860 | 1.16 (0.69-1.96) | 0.577 | 1.02 (0.60-1.75) | 0.942 |

[a] Adjusted for: gender, age, HIV-1 subtype, mode of HIV-1 transmission, year of cART initiation, pre-cART viral load, pre-cART CD4 cell count, type of initial regimen started, type of NRTI-backbone used, time to achieving VS and level of TDR detected at pre-cART GRT (calculated according to the list of Bennet et al., PLoS One 2009 [20]). [b] Reference group (dummy). A multiple imputation approach was performed to fill missing values. CI: confidence interval. cART: combined antiretroviral therapy. GRT: genotypic resistance test. HR: hazard ratio. NRTI: nucleos(t)ide reverse transcriptase inhibitors. TDR: transmitted drug resistance. VS: virological suppression. Boldface indicates factors that were significantly associated ($p<0.05$) with virological rebound.

**Supplementary Table 2. Factors associated with virological rebound in HIV-1 infected patients achieving virological suppression after the first-line therapy (by excluding 502 patients under a rilpivirine-containing regimen).**

| Variables | Hazard ratio of experiencing virological rebound (first of two consecutive plasma viral load >50 copies/mL) | | | | Hazard ratio of experiencing virological rebound (first of two consecutive plasma viral load >200 copies/mL) | | | |
|---|---|---|---|---|---|---|---|---|
| | Crude | | Adjusted[a] | | Crude | | Adjusted[a] | |
| | HR (95% CI) | p-value | HR (95% CI) | p-value | HR (95% CI) | p-value | HR (95% CI) | p-value |
| **Gender (*female* vs. *male*[b])** | **1.30 (1.11-1.52)** | **0.001** | 1.08 (0.92-1.27) | 0.358 | **1.64 (1.33-2.03)** | **<0.0001** | **1.26 (1.01-1.57)** | **0.043** |
| **Age (per 5 years increase)** | 1.02 (0.98-1.05) | 0.308 | 1.01 (0.97-1.04) | 0.653 | 0.96 (0.92-1.01) | 0.154 | 0.97 (0.92-1.02) | 0.246 |
| **HIV-1 subtype[c] (*non-B* vs. *B*[b])** | **1.39 (1.19-1.63)** | **<0.0001** | **1.50 (1.27-1.77)** | **<0.0001** | **1.48 (1.19-1.83)** | **<0.0001** | **1.65 (1.31-2.08)** | **<0.0001** |
| **Mode of HIV-1 transmission[c]:** | | | | | | | | |
| *Homosexual*[b] | 1 | | 1 | | 1 | | 1 | |
| *Heterosexual* | 1.10 (0.94-1.29) | 0.221 | 1.13 (0.97-1.32) | 0.124 | 1.21 (0.96-1.51) | 0.100 | 1.22 (0.97-1.53) | 0.089 |
| *Drug abuser* | **1.78 (1.44-2.19)** | **<0.0001** | **1.50 (1.21-1.86)** | **<0.0001** | **2.58 (1.96-3.39)** | **<0.0001** | **2.08 (1.57-2.75)** | **<0.0001** |
| *Other* | 1.02 (0.71-1.48) | 0.907 | 0.94 (0.65-1.36) | 0.726 | 0.82 (0.44-1.51) | 0.515 | 0.75 (0.40-1.38) | 0.355 |
| **Year of cART initiation (per 1 year increase)** | **0.90 (0.88-0.91)** | **<0.0001** | **0.89 (0.87-0.92)** | **<0.0001** | **0.86 (0.83-0.88)** | **<0.0001** | **0.88 (0.84-0.92)** | **<0.0001** |
| **Pre-cART viral load (copies/mL):** | | | | | | | | |
| *≤100,000*[b] | 1 | | 1 | | 1 | | 1 | |
| *100,001-500,000* | **1.22 (1.05-1.43)** | **0.010** | 1.16 (0.99-1.36) | 0.071 | 0.89 (0.71-1.11) | 0.296 | 0.91 (0.72-1.14) | 0.395 |
| *500,001-1,000,000* | **1.92 (1.48-2.50)** | **<0.0001** | **1.91 (1.44-2.52)** | **<0.0001** | **1.52 (1.03-2.22)** | **0.033** | **1.84 (1.23-2.75)** | **0.003** |
| *>1,000,000* | **2.18 (1.64-2.89)** | **<0.0001** | **1.96 (1.44-2.68)** | **<0.0001** | **1.76 (1.17-2.64)** | **0.007** | **1.87 (1.20-2.93)** | **0.006** |
| **Pre-cART CD4 cell count (per 100 cells/mm$^3$ increase)[c]:** | **0.85 (0.82-0.89)** | **<0.0001** | **0.94 (0.90-0.98)** | **0.007** | **0.89 (0.84-0.95)** | **<0.0001** | 0.96 (0.90-1.03) | 0.251 |
| **Type of initial regimen started:** | | | | | | | | |
| *2 NRTIs + 1 PIb*[b] | 1 | | 1 | | 1 | | 1 | |
| *2 NRTIs + 1 NNRTI* | **0.73 (0.63-0.85)** | **<0.0001** | **0.69 (0.59-0.81)** | **<0.0001** | 0.91 (0.74-1.11) | 0.350 | **0.79 (0.63-0.98)** | **0.031** |
| *2 NRTI + INI* | **0.46 (0.29-0.73)** | **0.001** | 0.87 (0.53-1.41) | 0.567 | **0.26 (0.10-0.69)** | **0.007** | 0.53 (0.19-1.44) | 0.210 |
| *PIb + INI + ≥1NRTI* | 0.65 (0.35-1.22) | 0.177 | 0.68 (0.33-1.39) | 0.294 | 0.63 (0.23-1.69) | 0.355 | 0.70 (0.22-2.25) | 0.555 |
| *Other* | 1.22 (0.79-1.87) | 0.364 | 0.90 (0.58-1.42) | 0.661 | 1.32 (0.74-2.36) | 0.352 | 0.83 (0.45-1.52) | 0.540 |
| **Type of NRTI-backbone used:** | | | | | | | | |
| *TDF + FTC*[b] | 1 | | 1 | | 1 | | 1 | |
| *ABC + 3TC* | 0.96 (0.71-1.29) | 0.772 | 0.89 (0.66-1.21) | 0.467 | 1.19 (0.78-1.82) | 0.417 | 1.10 (0.71-1.68) | 0.671 |
| *AZT + 3TC* | **2.05 (1.71-2.45)** | **<0.0001** | 1.09 (0.86-1.39) | 0.482 | **2.59 (2.02-3.31)** | **<0.0001** | 1.24 (0.89-1.74) | 0.205 |
| *Other* | **1.87 (1.51-2.30)** | **<0.0001** | 1.02 (0.77-1.36) | 0.873 | **2.80 (2.14-3.67)** | **<0.0001** | 1.32 (0.91-1.92) | 0.144 |
| **Time (months) to achieving VS:** | | | | | | | | |
| *<6*[b] | 1 | | 1 | | 1 | | 1 | |
| *6-12* | 1.08 (0.91-1.27) | 0.393 | 0.96 (0.80-1.14) | 0.606 | 0.85 (0.66-1.09) | 0.200 | 0.82 (0.63-1.06) | 0.137 |
| *>12* | **1.63 (1.33-2.01)** | **<0.0001** | 1.02 (0.81-1.27) | 0.890 | **1.59 (1.19-2.11)** | **0.002** | 0.92 (0.68-1.26) | 0.609 |
| **TDR detected at pre-cART GRT[c, d]** | 0.82 (0.65-1.03) | 0.091 | 0.84 (0.66-1.06) | 0.142 | 0.76 (0.54-1.07) | 0.116 | 0.83 (0.59-1.17) | 0.293 |

[a] Adjusted for: gender, age, HIV-1 subtype, mode of HIV-1 transmission, year of cART initiation, pre-cART viral load, pre-cART CD4 cell count, type of initial regimen started, type of NRTI-backbone used, time to achieving VS and level of TDR detected at pre-cART GRT. [b] Reference group (dummy). [c] A multiple imputation approach was performed to fill missing values. [d] As the presence of at least one mutation from WHO surveillance TDR list (Bennet et al, PLoS One 2009 [20]). 3TC: lamivudine. ABC: abacavir. AZT: zidovoudine. CI: confidence interval. cART: combined antiretroviral therapy. FTC: emtricitabine. GRT: genotypic resistance test. HR: hazard ratio. INI: integrase inhibitor. NNRTI: nucleoside reverse transcriptase inhibitor. NRTIs: nucleos(t)ide reverse transcriptase inhibitors. PIb: ritonavir-cobicistat boosted protease inhibitor. TDF: tenofovir. TDR: transmitted drug resistance. VS: virological suppression. WHO: world health organization. Boldface indicates factors that were significantly associated (p<0.05) with virological rebound.

**Supplementary table 3. Factors associated with virological rebound under the first-line therapy in HIV-1 infected patients achieving virological suppression (on treatment approach[a]).**

| Variables | Hazard ratio of experiencing virological rebound (first of two consecutive plasma viral load >50 copies/mL) | | | | Hazard ratio of experiencing virological rebound (first of two consecutive plasma viral load >200 copies/mL) | | | |
|---|---|---|---|---|---|---|---|---|
| | Crude | | Adjusted[b] | | Crude | | Adjusted[b] | |
| | HR (95% CI) | p-value | HR (95% CI) | p-value | HR (95% CI) | p-value | HR (95% CI) | p-value |
| **Gender (*female* vs. *male*[c])** | 1.21 (0.96-1.53) | 0.110 | 0.95 (0.75-1.22) | 0.696 | **1.63 (1.17-2.27)** | **0.004** | 1.17 (0.83-1.65) | 0.379 |
| **Age (per 5 years increase)** | 1.02 (0.97-1.08) | 0.359 | 1.00 (0.94-1.05) | 0.871 | 0.99 (0.92-1.07) | 0.887 | 0.98 (0.90-1.06) | 0.550 |
| **HIV-1 subtype[d] (*non-B* vs. *B*[c])** | **1.27 (1.02-1.58)** | **0.032** | **1.41 (1.12-1.77)** | **0.004** | **1.43 (1.03-1.97)** | **0.033** | **1.61 (1.14-2.27)** | **0.006** |
| **Mode of HIV-1 transmission[d]:** | | | | | | | | |
| *Homosexual*[c] | 1 | | 1 | | 1 | | 1 | |
| *Heterosexual* | 1.09 (0.87-1.36) | 0.471 | 1.14 (0.91-1.42) | 0.262 | 1.22 (0.87-1.72) | 0.247 | 1.22 (0.86-1.72) | 0.262 |
| *Drug abuser* | **2.05 (1.54-2.74)** | **<0.0001** | **1.72 (1.28-2.31)** | **<0.0001** | **2.69 (1.78-4.08)** | **<0.0001** | **2.09 (1.36-3.20)** | **0.001** |
| *Other* | 1.15 (0.71-1.85) | 0.577 | 1.16 (0.71-1.87) | 0.556 | 0.78 (0.31-1.94) | 0.594 | 0.76 (0.30-1.90) | 0.559 |
| **Year of cART initiation (per 1 year increase)** | **0.89 (0.86-0.91)** | **<0.0001** | **0.89 (0.85-0.93)** | **<0.0001** | **0.85 (0.82-0.89)** | **<0.0001** | **0.88 (0.82-0.94)** | **<0.0001** |
| **Pre-cART viral load (copies/mL):** | | | | | | | | |
| *≤100,000*[c] | 1 | | 1 | | 1 | | 1 | |
| *100,001-500,000* | **1.54 (1.24-1.90)** | **<0.0001** | **1.28 (1.03-1.60)** | **0.028** | 1.22 (0.88-1.69) | 0.242 | 1.12 (0.80-1.57) | 0.511 |
| *500,001-1,000,000* | **2.35 (1.61-3.44)** | **<0.0001** | **2.05 (1.37-3.07)** | **0.001** | **1.85 (1.01-3.38)** | **0.045** | **1.98 (1.05-3.73)** | **0.035** |
| *>1,000,000* | **2.36 (1.50-3.71)** | **<0.0001** | **1.92 (1.18-3.11)** | **0.008** | **2.12 (1.07-4.21)** | **0.032** | **2.10 (1.00-4.38)** | **0.049** |
| **Pre-cART CD4 cell count (per 100 cells/mm$^3$ increase)[d]:** | **0.79 (0.74-0.84)** | **<0.0001** | **0.89 (0.83-0.95)** | **0.001** | **0.80 (0.73-0.88)** | **<0.0001** | **0.89 (0.80-0.99)** | **0.032** |
| **Type of initial regimen started:** | | | | | | | | |
| *2 NRTIs + 1 PIb*[c] | 1 | | 1 | | 1 | | 1 | |
| *2 NRTIs + 1 NNRTI* | **0.63 (0.51-0.77)** | **<0.0001** | **0.64 (0.51-0.79)** | **<0.0001** | 0.87 (0.64-1.17) | 0.356 | 0.82 (0.59-1.14) | 0.239 |
| *2 NRTI + INI* | **0.39 (0.21-0.73)** | **0.004** | 0.78 (0.40-1.49) | 0.448 | **0.23 (0.06-0.94)** | **0.040** | 0.50 (0.12-2.06) | 0.336 |
| *PIb + INI + ≥1NRTI* | 0.68 (0.22-2.14) | 0.512 | 0.61 (0.15-2.49) | 0.490 | 0 (0-Inf) | 0.992 | 0 (0-Inf) | 0.991 |
| *Other* | 1.43 (0.67-3.04) | 0.351 | 1.00 (0.45-2.22) | 0.998 | 0.52 (0.07-3.76) | 0.519 | 0.27 (0.04-2.01) | 0.202 |
| **Type of NRTI-backbone used:** | | | | | | | | |
| *TDF + FTC*[c] | 1 | | 1 | | 1 | | 1 | |
| *ABC + 3TC* | 0.84 (0.54-1.29) | 0.421 | 0.83 (0.54-1.29) | 0.411 | 1.01 (0.53-1.94) | 0.971 | 1.01 (0.53-1.94) | 0.976 |
| *AZT + 3TC* | **2.23 (1.71-2.91)** | **<0.0001** | 1.03 (0.70-1.50) | 0.894 | **2.40 (1.60-3.58)** | **<0.0001** | 0.98 (0.55-1.74) | 0.950 |
| *Other* | **2.17 (1.58-2.98)** | **<0.0001** | 1.01 (0.66-1.55) | 0.970 | **3.51 (2.32-5.31)** | **<0.0001** | 1.45 (0.81-2.61) | 0.214 |
| **Time (months) to achieving VS:** | | | | | | | | |
| *<6*[c] | 1 | | 1 | | 1 | | 1 | |
| *6-12* | 1.10 (0.87-1.39) | 0.444 | 0.93 (0.73-1.19) | 0.583 | 0.77 (0.52-1.15) | 0.208 | 0.72 (0.47-1.09) | 0.120 |
| *>12* | **1.55 (1.09-2.20)** | **0.015** | 0.96 (0.66-1.38) | 0.807 | **1.81 (1.11-2.93)** | **0.016** | 1.09 (0.66-1.82) | 0.728 |
| **TDR detected at pre-cART GRT[d, e]** | **0.69 (0.49-0.99)** | **0.042** | **0.66 (0.46-0.95)** | **0.025** | **0.42 (0.22-0.83)** | **0.012** | **0.46 (0.23-0.91)** | **0.025** |

[a] Analysis performed by censoring patients at the end of their first-line regimen (N=4,509). [b] Adjusted for: gender, age, HIV-1 subtype, mode of HIV-1 transmission, year of cART initiation, pre-cART viral load, pre-cART CD4 cell count, type of initial regimen started, type of NRTI-backbone used, time to achieving VS and level of TDR detected at pre-cART GRT. [c] Reference group (dummy). [d] A multiple imputation approach was performed to fill missing values. [e] As the presence of at least one mutation from WHO surveillance TDR list (Bennet et al, 2009 [20]). 3TC: lamivudine. ABC: abacavir. AZT: zidovoudine. CI: confidence interval. cART: combined antiretroviral therapy. FTC: emtricitabine. GRT: genotypic resistance test. HR: hazard ratio. INI: integrase inhibitor. NNRTI: nucleoside reverse transcriptase inhibitor. NRTIs: nucleos(t)ide reverse transcriptase inhibitors. PIb: ritonavir-cobicistat boosted protease inhibitor. TDF: tenofovir. TDR: transmitted drug resistance. VS: virological suppression. WHO: world health organization. Boldface indicates factors that were significantly associated (p<0.05) with virological rebound.