

## **Burden of disease in PLWH harboring a multidrug resistant virus: data from PRESTIGIO Registry**

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Preliminary results of this work have been presented at the European AIDS Conference, November 6-9 2019, Basel, Switzerland. Abstract number PE20/11.

Accepted Manuscript

## **SUMMARY**

People living with HIV-1 infection harboring a four-class drug-resistance virus have a high burden of morbidity and mortality with approximately one in 4 developing a clinical event or death within 4 years; 32% of the events were malignancies.

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## ABSTRACT

**Background:** Currently, no data are available on the burden of morbidity and mortality in people living with HIV-1 (PLWH) harboring a 4-class drug resistant (4DR) virus (NRTI, NNRTI, PI, INSTI). The study aimed to assess the incidence of clinical events or death in this population.

**Methods:** Cohort study on PLWH, from the PRESTIGIO Registry, with a documented 4DR virus.

The burden of disease was defined as the occurrence of any new event including AIDS-defining event (ADE) or non-AIDS-defining event (NADE) or death for any cause, after 4DR evidence (baseline).

Cox regression models evaluated factors associated with the risk of new clinical events/death.

**Results:** Among 148 PLWH followed for a median of 47 months (IQR=32-84) since 4DR evidence, 38 PLWH had 62 new events or died for any cause [incidence rate, 9.12/100-PYFU (95%CI=6.85-11.39)]: 12 deaths (6 AIDS-related and 6 non-AIDS-related), 18 ADE, 32 NADE; 20 of the 38 NADE (45%) of the incident clinical events were malignancies. The 4-year cumulative incidence of death was 6% (95%CI=3%-13%) and that of  $\geq 1$  event or death was 22% (95%CI=16%-31%).

A higher risk of new clinical events/death was more likely in PLWH with previous clinical events (aHR=2.67, 95%CI=1.07-6.67) and marginally associated with lower baseline CD4+/CD8+ ratio (aHR=0.82, 95%CI=0.65-1.02).

**Conclusions:** PLWH harbouring 4DR have a high burden of disease with a worrying incidence of malignancies, strongly advising for close prevention and monitoring interventions as well as access to innovative therapeutic strategies, especially in people with a history of clinical events and low CD4+/CD8+ ratio.

**Keywords:** four-class drug-resistance, death, AIDS-defining event, non-AIDS-defining event, cancer.

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## INTRODUCTION

Since 2007, ART regimens based on an integrase strand transfer inhibitor (INSTI) have been introduced and largely used both in treatment-naïve and treatment-experienced people because of their safety and efficacy, as demonstrated in many clinical trials and observational studies [1-2]. The widespread use of the INSTI-based regimens, particularly first-generation agents, has led to the emergence of INSTI resistance in a limited proportion of people living with HIV-1 infection (PLWH) [3]. Italian data reported a 9% prevalence of multi-class drug resistance [MDR, defined as a virus with at least one major resistance mutation in at least 3 different drug classes [nucleoside reverse transcriptase inhibitors (NRTI), non-nucleoside reverse transcriptase inhibitors (NNRTI), protease inhibitors (PI), INSTI] in ART-experienced HIV-1 infected people in the period 2011-2018 [4]. The prevalence of four-class drug-resistance (4DR, i.e. with NRTI, NNRTI, PI, INSTI resistance) has recently arisen from 1% (2008-2013) to 3% and 2% (2014-2016 and 2017-2018, respectively) [4].

Previous studies suggested that people with three-class drug-resistance have a particularly poor prognosis with high morbidity and mortality. An Italian cohort study [5], including 623 PLWH and triple-class virological failure (TCVF), followed in 1999-2002 for a median follow-up of 21 months (IQR 7-37), reported an incidence rate (IR) of death for any cause equal to 11.9/100 person-years of follow-up (PYFU) and an IR of AIDS-related death of 9.4/100 PYFU. In addition, the 48-month probability of new AIDS event/death was estimated to be 35.9%.

In a previous European multi-cohort study [6] including PLWH with experienced TCVF, the estimated incidence rate of AIDS-defining events (1.2/100 PYFU in 2009) and the mortality (1.4/100 PYFU in 2008) did not decrease significantly in nine years of follow-up.

To date, however, data on the burden of AIDS-related and non-AIDS related morbidity in PLWH harboring a 4DR virus are not available. Then, using the PRESTIGIO Registry, the aim of this analysis was to evaluate the incidence of AIDS-related, non-AIDS-related events and death in this population.

## **METHODS**

The PRESTIGIO Registry ([www.registroprestigio.com](http://www.registroprestigio.com)) is an observational, prospective, Italian, multicenter, annual collection of data on clinical, laboratory, treatment and virological characteristics of PLWH and 4DR virus.

The registry includes people with the following characteristics: age >14 years; documented resistance to the 4 classes of antiretroviral drugs (NRTI, NNRTI, PI, INSTI), defined as intermediate or high-level resistance to at least one drug of each class according to the Stanford algorithm; if no INSTI genotypic resistance test is available, a documented virological failure to an INSTI-regimen is accepted as an inclusion criteria. The PRESTIGIO Registry is recorded in the ClinicalTrials.gov website with the following identifier: NCT04098315.

In this analysis, we included people from the PRESTIGIO Registry, with an available and verified clinical history and with  $\geq 1$  clinical visit after the date with the first evidence of a 4DR virus (baseline).

The outcomes for the present analysis were the incidence of: i)  $\geq 1$  new AIDS event or non-AIDS event or death for any cause; ii) death for any cause; iii)  $\geq 1$  new AIDS event or AIDS-related death; iv)  $\geq 1$  new non-AIDS event or non-AIDS-related death.

The AIDS events were defined according to the CDC's 1993 Revised Classification System for HIV Infection and Expanded Surveillance Case Definition for AIDS among Adolescents and Adult [7].



The non-AIDS events included: non-AIDS related malignancies, major adverse cardiovascular events (MACE), decompensated cirrhosis, chronic kidney disease (CKD) and diabetes. MACE included: acute myocardial infarction, coronary disease requiring invasive procedures, congestive heart failure and stroke. CKD was defined as the occurrence of two consecutive, at least three months apart, estimated glomerular filtrate rate determinations  $<60 \text{ mL/min/1.73m}^2$  (using the CKD Epidemiology Collaboration Equation formula).

All incident clinical events occurred since baseline until March 2019 (administrative censoring date) were considered in the analyses. Patients might have experienced a clinical event (AIDS-related or non-AIDS-related) prior to baseline.

#### ***Patient Consent Statement***

The protocol of the PRESTIGIO Registry was approved (protocol number 41/int/December 2017) by the San Raffaele Ethical Committee (coordinating center) in December 2017. Forty Infectious Diseases clinical centers, located in almost all the Italian regions, are actively participating and their Ethic Committees have also approved the registry protocol; all patients provided written informed consent to be enrolled.

#### ***Statistical analysis***

Results were described as median (interquartile range, IQR) and frequency (%).

Distributions of laboratory parameters were compared between PLWH with vs. without events using the Wilcoxon rank-sum test for continuous variables or the chi-square/Fisher exact test for categorical variables; we considered the nearest values to the date of the first event (within 90 days before the event), for people with events and the last available value, for people without events. Incidence rates (IR) were estimated

by univariate Poisson regression as number of events recorded after baseline and person years of follow-up (PYFU) and expressed as rate per 100 PYFU, with 95% confidence intervals (CI). As participants could experience more than one incident event, in measuring incidence, the occurrence of different types of clinical events was allowed while recurrences of the same type of event were excluded. In these analyses, follow-up started at BL and ended at the date of the incident clinical events, date of death, or the date of last available visit.

For time-to-event analyses, Kaplan-Meier curves were used to estimate the cumulative probability of the first incident: i) clinical event (AIDS- or non-AIDS-related or death for any cause, whichever occurred first); ii) AIDS-related event or AIDS-related death; iii) non-AIDS-related event or non-AIDS-related death after 4DR evidence. In these analyses, follow-up started at baseline and ended at the date of first event, date of death or last available visit (whichever occurred first) and was right-censored 96 months after baseline due to the low number of cases observed thereafter; there were not competing events.

Time to the first clinical event (either AIDS- or non-AIDS-related) or death for any cause was estimated by use of the Kaplan-Meier curves.

Multivariable Cox regression models were fitted to determine the factors associated with the risk of  $\geq 1$  clinical event (either AIDS- or non-AIDS-related) or death for any cause; the effect estimate was reported as the adjusted hazard ratio (aHR) with the corresponding 95% CI, estimated according to the Wald approximation. The multivariable model included covariates with a p-value  $< 0.2$  in univariable analysis. To define the final multivariable model, we fitted different models and assessed for

collinearity. The multivariable models included the correlated variables, first individually and then in combination; the additional contribution of each covariate was assessed with the Akaike's information criterion. The variables included in the models were fitted as time-fixed and measured at baseline.

For the analyses, two-sided p-values <0.05 were considered statistically significant. All analyses were performed using the SAS Software, release 9.4 (SAS Institute, Cary, NC).

## RESULTS

Overall, 148 PLWH harboring a 4DR virus were evaluated and contributed to 680 PYFU. They were predominantly males (78%), with a median age of 49 years (IQR 44-53), 21% co-infected with HCV, treated with ART for 16.8 years (IQR 14.0-19.7). Thirty-nine percent had experienced previous AIDS events and 21% had previous non-AIDS events, mostly a non-AIDS malignancy (16%); at baseline, the RGSS score was 5.5 (IQR 3.75-9.5), 93% of people had plasma HIV-1 RNA >50 copies/mL with a median of 3.49 log<sub>10</sub> copies/mL (IQR 2.37-4.48), CD4+ cell count was 346 cells/μL (IQR 169-600), and CD4+/CD8+ ratio was 0.35 (IQR 0.20-0.61). Other characteristics of the 148 people considered in this study are provided in Table 1.

During a median follow-up of 47 months (IQR 32-84) after the first 4DR evidence, 38 PLWH had 62 new events or died for any cause [12 deaths for any cause (6 AIDS-related deaths and 6 non-AIDS-related deaths), 17 primary non-AIDS-defining malignancies, 3 primary AIDS-defining malignancies, 15 AIDS events, 6 MACE, 6 CKD and 3 cirrhosis].

The incidence rate of any new event or death for any cause was 9.12/100-PYFU (95%CI 6.85-11.39). Fifteen PLWH developed 24 new AIDS events or had AIDS-related death [IR

3.52/100-PYFU, 95%CI 2.12-4.94]; the incidence rate of AIDS-related events was 2.65/100-PMFU (95%CI 1.57-4.01). Twenty-six PLWH developed 38 non-AIDS events or had non-AIDS-related death [IR 5.59/100-PYFU, 95%CI 3.81-7.37]; the incidence rate of non-AIDS-related events was 4.71/100-PMFU (95%CI 3.08-6.34).

Interestingly, 20 (32% in 16 PLWH) of the incident clinical events were malignancies and, among the incident non-AIDS events, 17 (45%) were non-AIDS-defining malignancies. Among the 38 people who had a first primary malignancy (22 before, 14 after and 2 both before and after the date of 4DR evidence), 1 (3%) individual had a relapse of the same malignancy (primary diagnosis of Hepatocellular carcinoma in 2016 with relapse in 2018) and 4 (11%) individuals had a second diagnosis of cancer: 1 anal cancer in 2013 (before 4DR date) followed by a cutaneous Kaposi sarcoma (KS) in 2019; 1 cutaneous KS in 1997 (before 4DR date) followed by a squamous cell skin cancer in 2016; 1 Hodgkin lymphoma in 2010 followed by a Non-Hodgkin lymphoma in 2017; 1 Hodgkin lymphoma in 2014 followed by a lung cancer in 2017. At the time of this study, 4/38 (11%) had died.

Kaplan Meier analysis showed that the estimated probabilities of a clinical event (AIDS event or non-AIDS event or death for any cause, whichever occurred first) were 10% (95%CI 6%-16%), 13% (95%CI 9%-20%), 17% (95%CI 11%-24%) and 22% (95%CI 16%-31%) at 12, 24, 36, 48 months since BL, respectively. People with incident clinical events or death for any cause, compared to those without events/death, had lower baseline CD4+ cell counts [210 (IQR 49-443) vs. 436 cells/ $\mu$ L (IQR 223-629);  $P=0.003$ ], lower baseline CD4+/CD8+ ratio [0.20 (IQR 0.09-0.44) vs. 0.45 (IQR 0.24-0.69);  $P=0.002$ ] and RGSS scores marginally lower [4.8 (IQR 2.5-7.5) vs. 6 (IQR 4-10);

$P=0.072$ ]. Baseline HIV-1 RNA was  $>50$  copies/mL in 95% of people with incident clinical events/death and in 92% of people without events/death ( $P=0.792$ ).

Overall, the incidence rate of death for any cause was 1.76/100-PYFU (95%CI 0.91-2.90). The cumulative probabilities of death were 1.4% (95%CI 0.4%-5.6%), 2.2% (95%CI 0.7%-6.7%), 3.0% (95%CI 1.1%-7.9%) and 6.2% (95%CI 3.0%-12.8%) at 12, 24, 36, 48 months since BL, respectively (Figure 1).

Twelve months after baseline, 4% (95%CI 2%-9%) of PLWH were estimated to have developed AIDS event or AIDS-related death, increasing to 6% (95%CI 3%-11%), 7% (95%CI 4%-12%) and 9% (95%CI 5%-15%) at 24, 36, 48 months, respectively (Figure 1). People with incident AIDS events/death, compared to people without AIDS events/death, had a similar baseline virological profile (HIV-1 RNA  $>50$  copies/mL: 100% vs 92%,  $P=0.604$ ) and a poor immunological profile [CD4+ cell count: 55 (IQR 20-328) vs. 416 cells/ $\mu$ L (IQR 204-629),  $P=0.0001$ ; CD4+/CD8+ ratio: 0.10 (IQR 0.06-0.24) vs. 0.38 (IQR 0.22-0.69),  $P=0.0006$ ]. At the event occurrence or last available visit, HIV-1 RNA  $>50$  copies/mL was recorded in 70% of people with incident AIDS events/death vs. 24% of people without AIDS events/death ( $P=0.004$ ) and a poor immunological profile was significantly associated with the occurrence of incident AIDS events or AIDS-related death (Figure 2).

The estimated probabilities of non-AIDS events or non-AIDS-related deaths were 5% (95%CI 3%-10%), 8% (95%CI 5%-15%), 12% (95%CI 7%-19%) and 15% (95%CI 10%-24%) at 12, 24, 36, 48 months since BL, respectively (Figure 2). People with incident non-AIDS events/death, compared to people without non-AIDS events/death,

had a similar baseline virological profile (HIV-1 RNA >50 copies/mL: 92% vs 93%,  $P=0.994$ ) and immunological profile [CD4+ cell counts: 282 (IQR 168-501) vs. 354 cells/ $\mu$ L (IQR 170-601),  $P=0.417$ ; CD4+/CD8+ ratio: 0.26 (IQR 0.15-0.54) vs. 0.37 (IQR 0.21-0.61),  $P=0.266$ ]. On the contrary, at the event occurrence or last available visit, people with incident non-AIDS events or non-AIDS-related death more frequently had HIV-1 RNA >50 copies/mL [59% of people with events vs. 24% of people without events;  $P=0.002$ ] and a poor immunological profile (Figure 3).

The estimated probabilities of any clinical event (AIDS event or non-AIDS event) or death for any cause, AIDS event or AIDS-related death, non-AIDS events or non-AIDS-related deaths, according to according to age (<50 vs  $\geq$ 50 years), baseline CD4+ cell count ( $\leq$ 350 vs >350 cells/ $\mu$ L) and baseline viral load (<50 vs  $\geq$ 50 copies/mL and  $\leq$ 200 vs >200 copies/mL) are reported in the Supplementary Material (Figure S1-S12). Age was associated only with the occurrence of non-AIDS-related events or non-AIDS-related death while baseline CD4+ cell count influenced either the burden of disease or the occurrence of AIDS-related events or AIDS-related death; viral load stratified on the basis of both cut-offs was not found to be associated to any of the study outcomes.

At multivariate analysis (Table 2), the occurrence of previous clinical events (AIDS or non-AIDS) before baseline was associated with an increased risk of incident clinical events/death [aHR ( $\geq$ 1 vs none) 2.674, 95%CI 1.067-6.667;  $P=0.036$ ] while higher values of BL CD4+/CD8+ ratio were marginally associated with a lower risk of incident clinical events/death for any cause [aHR (per 0.1-unit higher) 0.816, 95%CI 0.654-1.019;  $P=0.072$ ].

## DISCUSSION

In this analysis, we reported that a substantial proportion of the 148 HIV-1 adults with 4DR had new clinical events or death following 4DR evidence, mainly non-AIDS-related (61%); 32% of the recorded clinical events were malignancies and, among the non-AIDS events, 45% were non-AIDS-defining malignancies. The occurrence of new clinical events or death for any cause was affected by the immunological profile at 4DR and marginally by a history of clinical events.

While the implication of multidrug resistance on clinical progression has been previously studied in people with TCVF [5-6, 8-9], no data have been yet reported in HIV-infected people with 4DR. Data from the PLATO II project [6], including 91764 individuals, showed that the crude incidence of AIDS-defining events was 1.2/100 PYFU in 2009 and the crude rate of death in 2008 was 1.4/100 PYFU. These rates were derived around ten years earlier than our study, when treatment options were less effective. However, the rates of AIDS-related events and death in our 4DR cohort are definitely higher (2.65 and 1.76/100-PYFU, respectively). Although the estimates obtained from the PRESTIGIO Registry derive from a smaller sample size, our findings highlight an excess of clinical events and mortality among PLWH and 4DR virus, despite the availability of modern and highly effective antiretroviral drugs.

Another relevant finding from our study is the high incidence of non-AIDS events or non-AIDS-related death, with a rate much higher than that of the AIDS-related events. Similar findings were previously observed in a study on 12844 PLWH by Mocroft A. et al. [10] reporting that non-AIDS events are common among PLWH in the HAART era,

associated with considerably mortality and that there is no relationship between the incidence of these events with CD4+ levels, especially above 350 cells/ $\mu$ L; the authors also observed that the incidence of non-AIDS events was similar to, or exceeded, the incidence of AIDS events at CD4 counts  $\geq 200$  cells/ $\mu$ L. We indeed estimated a higher incidence rate of non-AIDS events in our study likely reflecting the higher degree of fragility of people with 4DR population compared to PLWH population in general.

In our study, one third of these events were malignancies (45% among the incident non-AIDS events), at least in part virus-related cancers (EBV-, HPV- and HCV-related), followed by cardiovascular events and that the occurrence of these events or death is more likely in people with a history of previous clinical events (48% of people had  $\geq 1$  clinical event before the evidence of 4DR). Most of the observed non-AIDS events are similar to those experienced by the general population, and, at least in part, potentially associated with modifiable lifestyle factors, such as smoking.

It is also of interest to note that, in our study, among the 24 people who survived a first primary malignancy, 4 (17%) individuals had a diagnosis of a second primary cancer. Although the limited sample size of our study might have negatively affected the accuracy of this estimate, this proportion slightly exceeds that estimated in a recent population study by Hessol NA and colleagues [11] showing that 9% of all the identified primary cancers among people with HIV were second or later primary cancers, a proportion similar to that in the general population of people aged 20-64 years (11%) [12]. Tenorio and colleagues, reported that, in people on suppressive ART, inflammation and coagulation markers are associated with higher risk of non-AIDS-defining events and mortality [13]. Borges and colleagues [14] also found that activated inflammation and coagulation pathways are associated with increased cancer risk during HIV infection. We may argue that these conditions are even truer for people with a 4DR



virus, frequently characterized by persistent viremia (possibly associated with non-adherence) and likely affected by a higher burden of inflammation [15].

Another finding from this study is that low CD4<sup>+</sup>/CD8<sup>+</sup> ratio values are marginally associated with a higher risk of new clinical events or death. Low CD4<sup>+</sup>/CD8<sup>+</sup> ratio cell counts have long been established as a risk factor for the development of clinical events and death, either AIDS-related [16] or non-AIDS related [17-19]. In consideration of the fact that most of the non-AIDS related events in our study were malignancies, the study by Hema et al. [19] is of particular interest; in fact, the authors reported that the risk of cancer was twice higher in the subset of patients who had a CD4<sup>+</sup>/CD8<sup>+</sup> ratio below 0.5, independently of CD4<sup>+</sup> cells count. This finding is in line with the median CD4<sup>+</sup>/CD8<sup>+</sup> ratio we observed in people with 4DR virus either at baseline or at the event occurrence.

There are some limitations in our study that are important to highlight. Although, clinical records were carefully monitored, we cannot exclude a potential underestimation of clinical events, especially of non-AIDS-related events and mortality for which a link with the national death registry is not available in Italy. Another issue is related to the recent establishment of the PRESTIGIO Registry: recorded people are those who survived a previous potentially fatal event and, for this reason, we might have underestimated the true incidences of AIDS-, non-AIDS-related events and death.

The absence of a control group is an additional study limitation that impairs the assessment of whether the estimated incidence rates are similar, less than or greater than those observed in other individuals. Unfortunately, a study that evaluated the same outcomes in individuals with similar characteristics and without extensive drug

resistance is lacking and, at this time, any comparator group can only be invariably inadequate. Another limitation relies on the date we considered as baseline for these analyses: this is the date on which four-drug resistance was highlighted for the first time, evidence that might be late compared to the time when this condition actually occurred. Finally, predictive factors were estimated on the composite endpoint including AIDS-, non-AIDS-related events and death for any cause; this analysis allowed to increase power although the potential risk factors may differ for each single component of the composite endpoint.

In conclusion, among PLWH and 4-class drug resistant virus, the occurrence of clinical events or death for any cause is particularly high and the incidence of new non-AIDS events/non-AIDS-related death almost twice as that of new AIDS events/AIDS-related death, with a worrying incidence of malignancies. Because of the high burden of disease in PLWH with 4DR virus, close prevention and monitoring interventions are highly recommended, especially in people with a history of clinical events and low CD4+/CD8+ ratio. In PLWH with 4DR virus, efforts to favor access to drugs with new mechanisms of action are also urgently needed in order to achieve undetectable HIV-RNA load, preserve immune system, prevent clinical progression and ultimately improve quality of life.

## NOTES

### Author contributions

A.C., L.G. and A.P. had full access to all the data in the study and take final responsibility for the integrity of the data, the accuracy of the data analysis and for the decision to submit for publication. Study concept: A.C. Data collection, administrative or material support: A.P., MR.P., M.M., M.F., E.G., D.F., A.DB., G.S., C.F., A.DA., G.A., FM.F., A.DM., GM.C., MM.S., M.Z. Statistical analysis: L.G., A.P. Drafting of the manuscript: L.G., MR.P., A.C. Data interpretation and critical revision of the manuscript: all authors.

### Funding

This work and the PRESTIGIO Registry were supported by ViiV Healthcare and Theratechnologies. The funding source had no involvement in the study.

This retrospective, longitudinal study was approved by the Ethics Committee of the San Raffaele Scientific Institute (approval No. 34-2017) and evaluated data from the HIV Clinic of

San Luigi Center, San Raffaele Hospital, in Milan, Italy.

### Potential conflicts of interests

All authors report no potential conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

### Acknowledgements

The authors wish to thank the PRESTIGIO Study Group:

STEERING COMMITTEE: Antonella Castagna (Coordinator), Nicola Gianotti, Laura Galli, Franco Maggiolo, Leonardo Calza, Emanuele Focà, Gaetana Sterrantino, Giovanni Cenderello, Antonio Di Biagio, Stefano Rusconi, Cristina Mussini, Marianna Menozzi, Andrea Antinori,

Roberta Gagliardini, Stefano Bonora, Micol Ferrara, Maurizio Zazzi, Maria Santoro, Giulio Maria Corbelli.

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Table 1. Baseline<sup>a</sup> characteristics of the 148 PLWH and 4-class drug resistant virus

Baseline characteristics	Median (IQR) or frequency (%)
Age (years)	49 (44-53)
Male gender	115 (78%)
HCVAb positive	31 (21%)
HBsAg positive	13 (9%)
Years from HIV diagnosis	20.4 (16.2-24.5)
Years of ART	16.8 (14.0-19.7)
Pre-ART plasma HIV-RNA ( $\log_{10}$ copies/mL)	5.18 (4.95-5.67)
Nadir CD4+ cell count (cells/ $\mu$ L)	92 (18-203)
Previous AIDS events	58 (39%)
Previous non-AIDS events	31 (21%)
Diabetes	9 (6%)
Decompensated cirrhosis	1 (1%)
Malignancies	24 (16%)
MACE	4 (3%)
CKD (eGFR <60 mL/min/1.73m <sup>2</sup> )	3 (2%)
Number of available Genotypic Resistance Tests per individual	3 (2-6)
Residual genotypic susceptibility score (RGSS score) <sup>b</sup>	5.5 (3.75-9.5)
NRTI RGSS	0.5 (0-2)

NNRTI RGSS	1 (0.5-2.5)
PI RGSS	1 (0-4)
INSTI RGSS	1 (0.5-2)
Previous exposure to maraviroc	50 (34%)
Previous exposure to enfurvitide	56 (38%)
Most frequent ART regimens	
DRV/b+DTG	21 (14.2%)
DRV/b+DTG+MVC	9 (6.1%)
DRV/b+DTG+ETV	8 (5.4%)
FTC/TAF+DRV/b+DTG	8 (5.4%)
3TC+DRV/b+DTG	6 (4.1%)
DRV/b+DTG+RPV	5 (3.4%)
InSTI-including regimen	128 (86.5%)
Plasma HIV-1 RNA $\geq 50$ copies/mL	137 (93%)
Plasma HIV-RNA ( $\log_{10}$ copies/mL)	3.49 (2.37-4.48)
CD4+ cell count (cells/ $\mu$ L)	346 (169-600)
CD8+ cell count (cells/ $\mu$ L)	946 (629-1284)
CD4+/CD8+ ratio	0.35 (0.20-0.61)

Abbreviations: ART, antiretroviral therapy; MACE, major adverse cardiovascular events; chronic CKD, kidney disease; eGFR, estimated glomerular filtration rate; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; INSTI, integrase strand transfer inhibitor.

<sup>a</sup>Baseline is defined as the date with the first evidence of a 4DR virus.

<sup>b</sup>The “Residual genotypic susceptibility score” (RGSS).was estimated according to the cumulative data of the available plasma genotyping resistance tests (GRT) recorded for each patient until baseline. Based on the patient’s cumulative resistance mutations, the level of resistance of each of 24 antiretroviral drugs (excluding maraviroc, enfuvirtide) was calculated using the Genotypic Resistance Interpretation Algorithm of the Stanford HIV Drug Resistance database Program (version 8.8, last updated on 2019-02-13; <http://hivdb.stanford.edu>). For each of the 24 FDA-approved antiretroviral drugs, a drug penalty score was assigned according to the degree of resistance. The antiretroviral drug resistance was scored as 1 point if classified as “Susceptible” or “Potential Low-Level or Low-Level Resistance”, as 0.5 point if classified as “Intermediate Resistance”, as 0 point if classified as “High-Level Resistance”. In people with a documented virological failure to an INSTI-regimen (n=21; 11 individuals failed raltegravir, 8 dolutegravir and 2 elvitegravir), the antiretroviral drug resistance (of the failed drug) was scored as 0.5 point; similarly, people previously failing maraviroc (n=50) or enfuvirtide (n=56) containing regimens were labeled as resistant to these agents and scored as 0.5 point.

Table 2. Univariable and multivariable hazard ratios for incident clinical events or death for any cause among the 148 PLWH and 4-class drug resistant virus

Covariate	Category	Univariable analysis		Multivariable analysis <sup>a</sup>	
		Hazard ratio (95% CI)	<i>P</i> Value	Adjusted Hazard ratio (95% CI)	<i>P</i> Value
Age	Per 1 year older	1.036 (0.993-1.080)	0.102	1.022 (0.972-1.073)	0.397
Gender	Female vs male	0.546 (0.210-1.418)	0.194	0.773 (0.264-2.262)	0.639
HCVAb	Positive vs negative	1.227 (0.546-2.758)	0.621	Not included	-
HBsAg	Positive vs negative	2.856 (0.377-21.634)	0.310	Not included	-
Years since HIV diagnosis	Per 1 year longer	1.003 (0.906-1.024)	0.230	Not included	-
Years of ART	Per 1 year longer	0.967 (0.898-1.041)	0.369	Not included	-
Pre-ART HIV-RNA	Per 1 log <sub>10</sub> copies/mL higher	0.628 (0.181-2.178)	0.464	Not included	-
Nadir CD4+ cell count	Per 100 cells/ $\mu$ L higher	0.531 (0.346-0.816)	0.004	0.756 (0.487-1.173)	0.212
Previous AIDS events	$\geq 1$ vs none	2.313 (1.141-4.688)	0.020	Not included	-
Previous non-AIDS event	$\geq 1$ vs none	2.724 (1.327-5.593)	0.006	Not included	-
Previous AIDS or non-AIDS event	$\geq 1$ vs none	3.644 (1.760-7.547)	0.0005	2.674 (1.067-6.667)	0.036

BL plasma HIV-1 RNA	≥50 vs <50 copies/mL	2.209 (0.300-16.250)	0.436	Not included	-
BL plasma HIV-1 RNA	Per 1 log <sub>10</sub> copies/mL higher	1.299 (0.967-1.745)	0.083	0.992 (0.615-1.600)	0.973
BL CD4+ cell count	Per 100 cells/μL higher	0.802 (0.687-0.936)	0.005	Not included	-
BL CD8+ cell count	Per 100 cells/μL higher	1.031 (0.965-1.101)	0.363	Not included	-
BL CD4+/CD8+ ratio	Per 0.1 unit higher	0.729 (0.602-0.885)	0.001	0.816 (0.654-1.019)	0.072
Burden of resistance (RGSS score)	≤5.5 vs >5.5 <sup>b</sup>	1.630 (0.830-3.204)	0.176	Not included	-
Burden of resistance (RGSS score)	Per 1-point higher	0.962 (0.882-1.049)	0.179	1.051 (0.936-1.180)	0.400

Abbreviations: CI, confidence interval; ART, antiretroviral therapy; BL, baseline (date with the first evidence of a 4DR virus); RGSS, residual genotypic susceptibility score.

<sup>a</sup>Covariates with univariable p value < 0.20 were included in the final multivariable model.

<sup>b</sup>Median value

## FIGURE LEGENDS

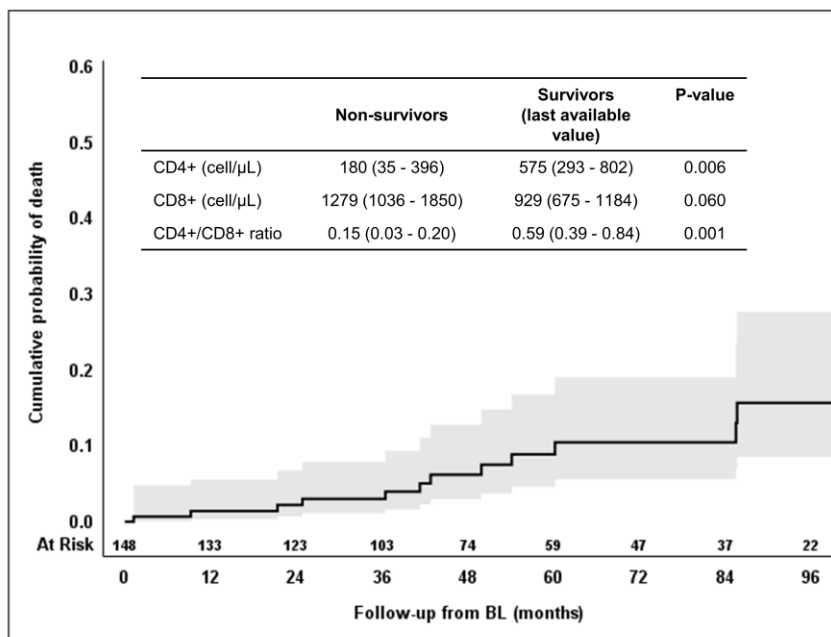
Figure 1. Time to death for any cause.

Figure 2. Time to occurrence of new AIDS-related events or AIDS-related death.

Figure 3. Time to occurrence of new non-AIDS-related events or non-AIDS related death.

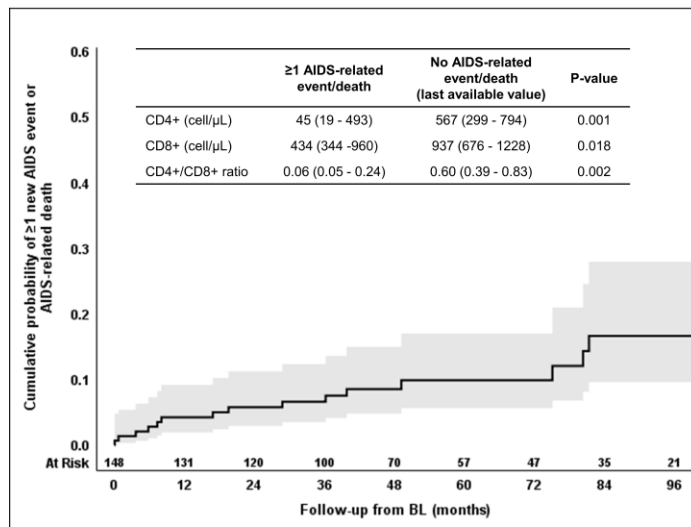
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Figure 1: Time to death for any cause



Twelve PLWH died [IR 1.76/100-PYFU, 95%CI 0.91-2.90]: 6 AIDS-related deaths [1 wasting syndrome, 1 Non-Hodgkin lymphoma (Burkitt), 1 tuberculous meningoencephalitis, 1 *Pneumocystis jirovecii*, 1 Mycobacteriosis, 1 extra-pulmonary *Mycobacterium tuberculosis*] and 6 non-AIDS related deaths [2 MACE, 1 Hodgkin lymphoma, 1 bacterial pneumonia, 1 suicide, 1 unspecified non-AIDS event].

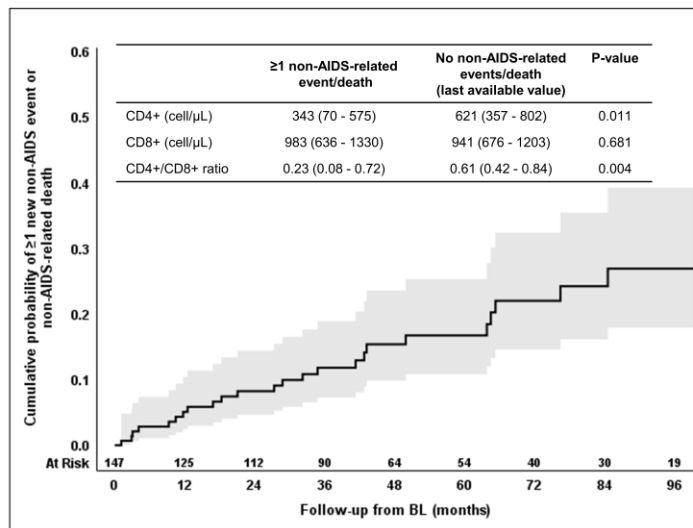
Figure 2. Time to occurrence of new AIDS-related events or AIDS-related death



Fifteen PLWH developed 24 new AIDS events or had AIDS-related death [IR 3.52/100-PYFU, 95%CI 2.12-4.94]: 3 atypical mycobacteriosis, 3 pulmonary tuberculosis, 1 tuberculous meningoencephalitis, 2 Non-Hodgkin lymphoma, 1 cutaneous Kaposi sarcoma, 2 esophageal candidiasis, 2 Pneumocystis jirovecii pneumonia, 1 disseminated Herpes simplex, 1 wasting syndrome, 1 cryptococcal meningitis, 1 cytomegalovirus encephalitis and 6 AIDS related deaths.



Figure 3: Time to occurrence of new non-AIDS-related events or non-AIDS related death



Twenty-six PLWH developed 38 non-AIDS events or had non-AIDS-related death [IR 5.59/100-PYFU, 95%CI 3.81-7.37]: 3 Hodgkin lymphoma, 4 anal cancer, 3 Hepatocellular carcinoma, 2 prostate cancer, 1 lung cancer, 1 breast cancer, 1 laryngeal cancer, 1 conjunctival spinocellular carcinoma, 1 squamous cell skin cancer, 6 MACE, 6 CKD, 3 cirrhosis and 6 non-AIDS related deaths.