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Summary

We documented a high prevalence of pretreatment drug resistance (PDR) amongst participants enrolled in trial clinics in rural KwaZulu-Natal. Dual class PDR to first-line tenofovir/emtricitabine/efavirenz regimen was associated with poorer VS. However, there was no impact of NNRTI PDR alone.

Abstract

Background

Previous studies in HIV-positive individuals on thymidine analogue backbone antiretroviral therapy (ART) with either nevirapine or efavirenz have suggested poorer virological outcomes in the presence of pretreatment drug resistance (PDR). We assessed the impact of PDR on virological suppression (VS) [<50copies/mL] in individuals prescribed primarily tenofovir/emtricitabine/efavirenz in rural KwaZulu-Natal within a Treatment as Prevention trial.

Methods

Among 1,557 HIV-positive individuals reporting no prior ART at study entry and provided plasma samples, 1,328 individuals with entry viral load (VL) >1,000 copies/mL had next generation sequencing (NGS) of the HIV *pol* gene with MiSeq technology. Results were obtained for 1,148 individuals and the presence of PDR assessed at 5% and 20% detection thresholds. Virological outcome was assessed using Cox regression in 837 of 920 ART initiators with at least one follow-up VL after ART initiation.

Results

PDR prevalence was 9.5% (109/1,148) and 12.8% (147/1,148) at 20% and 5% thresholds respectively. After a median of 1.36years (IQR 0.91-2.13), mostly on fixed-dose combination (FDC) tenofovir/emtricitabine/efavirenz, presence of both NRTI/NNRTI PDR vs. no PDR was associated with longer time to VS [aHR 0.32, 95%CI=0.12-0.86] while there was no difference between those with only NNRTI PDR vs. no PDR [aHR 1.05, 95%CI=0.82-1.34] at the 5% threshold. Similar differences were observed for mutations detected at the 20% threshold, although without statistical significance.

Conclusions

NGS uncovered a high prevalence of PDR amongst participants enrolled in trial clinics in rural KwaZulu-Natal. Dual class PDR to a mainly tenofovir/emtricitabine/efavirenz was associated with poorer VS. However, there was no impact of NNRTI PDR alone.

Keywords: HIV, pretreatment drug resistance, antiretroviral therapy, next-generation sequencing, virological response, sub-Saharan Africa

Introduction

HIV antiretroviral therapy (ART) scale-up in Eastern and Southern Africa has been a great success, with a doubling of the number of people on ART since 2010, reaching 10.3 million people in 2016 with a 36% decline in the number of AIDS-related deaths [1]. Despite the benefits of ART for individuals and populations [2, 3], expanding ART access and longer time on therapy might increase emergence and transmission of drug resistance (DR) [4], which could potentially compromise public ART programmes in settings using standardized first-line regimens. The majority of studies in SSA (Supplementary Table 1) have shown a detrimental impact of pre-treatment DR (PDR) on virological outcomes in individuals prescribed first-line ART mainly comprising a thymidine analogue backbone (zidovudine [ZDV] or stavudine [d4T] combined with either efavirenz (EFV) or nevirapine (NVP) [4-9]. Four of these studies accounted for ART adherence [4-6, 8]. Fewer, generally smaller sized studies, studying populations prescribed mainly older first-line ART regimen, have not shown a similar association [10-13].

Within the Treatment as Prevention (TasP) trial, a cluster-randomised trial undertaken in an HIV hyper-epidemic setting in rural KwaZulu-Natal, South Africa [14], we estimated the prevalence of PDR using next generation sequencing (NGS) technologies amongst HIV-positive participants who reported not to be on ART at entry into trial clinics. We evaluated the association between PDR, and response to first-line ART (predominantly fixed-dose combination (FDC) tenofovir/emtricitabine/efavirenz (TDF/FTC/EFV) (Atripla®)) in individuals who initiated ART within the trial.

Methods

Ethics statement

The trial was approved by the Biomedical Research Ethics Committee (BFC 104/11) at the University of KwaZulu-Natal and the Medicines Control Council of South Africa (Clinicatrials.gov: NCT01509508; South African National Clinical Trials Register: DOH-27-0512-3974). All trial participants gave written or witnessed thumbprint informed consent prior to undertaking any study procedures.

Study Design and Trial Setting

The ANRS 12249 TasP trial was implemented in the Hlabisa sub-district in rural KwaZulu-Natal [14], one of the poorest communities in South Africa, with high unemployment rate [15]. This was a cluster-randomised trial undertaken between March 2012 and June 2016 in 22 clusters (2 x11) [16, 17]; participants residing in the intervention clusters were offered ART after HIV diagnosis, regardless of their CD4 count, whereas participants in control clusters were offered ART according to the prevailing South African guidelines.

Study Procedures and Laboratory Methods

Individuals 16 years and above testing HIV-positive through home-based rapid test or who self-reported to be HIV-positive were referred to the trial clinics in their cluster, regardless of their ART status.

Individuals who linked to care were asked to complete study questionnaires and provide plasma samples at their first trial clinic visit, then at three months, six months and six monthly thereafter, if they initiated ART. Plasma samples were used for viral load (VL) testing, using the Abbott RealTime HIV-1 m2000rt (Abbott Molecular Inc, Des Plaines, IL, USA), as well as for DR testing in the Africa Health Research Institute diagnostic laboratory. Individuals visited the clinics monthly for their ART prescription, where adherence was measured using the visual analogue scale (VAS) [18]. Participants were asked to mark their level of adherence in the previous 4 days on a visual analogue scale ranging from 0 (no ART tablets taken) to 100% (all ART tablets taken). Adherence was suboptimal if ≤95%.

Plasma samples with VL≥1,000 copies/mL were characterised for HIV *pol* with NGS, using MiSeq technology, according to an adapted protocol from Gall et al (Supplementary Methods 1 and Supplementary Table 2) [19]. After reads assemblies using Geneious 10.0.6 software [20] and quality control of NGS data, DR mutations (DRM) were called at a threshold of 5% (Supplementary Methods 2). Resistant variants were included in the analysis when they were also detected by another application available in BaseSpace MiCall [21]. The DRM were documented using the WHO 2009 surveillance of DRM [22]; PDR prevalence and impact were estimated from DRM detected at >5%, confidence level of real mutation detection, and >20%, level of detection reached by Sanger population sequencing, most common technique used in DR testing.

Statistical Analysis

The characteristics of individuals who had NGS sequence data at baseline with and without PDR were tabulated. Characteristics of individuals who initiated ART in the trial, had NGS sequence data at baseline, and had at least one follow-up VL measurement (i.e. so included in the analysis of VS) were tabulated and compared with those of individuals who were missing VL at follow-up. We checked for completeness of VL measurements in those with and without PDR during the first 12 months after ART initiation to exclude ascertainment bias.

Categorical variables were summarised using frequencies and proportions, and compared using Chi-squared tests. Continuous variables were summarised using median and interquartile ranges (IQR), and compared using Mann-Whitney tests.

We computed the overall proportions of individuals with any PDR and NNRTI at 5% and 20% detection thresholds. We examined the association between PDR stratified based on predicted response to the antiretroviral drugs prescribed (no PDR, only NNRTI PDR or both NRTI/NNRTI PDR), and time to VS. Two separate analyses were undertaken for time to VS; PDR was defined as whether or not mutations were present at the 20% threshold, and then at the 5% threshold. Kaplan Meier methods were used to estimate time to VS in the three PDR categories, which were compared using the log rank test. Individuals entered the analysis at the date of ART initiation; those who did not achieve VS were censored at the date of their last VL measurement. Cox regression was used to estimate hazard ratios (HR) and 95% confidence intervals (CI) for the association of PDR and other factors with VS. Factors that were associated with VS at $p<0.15$ in the unadjusted analysis were included in a multivariable model; age and sex were retained a priori as potential confounders. CD4 count and age were included in the model as continuous covariates. In order to allow for a non-linear relationship between CD4 count, age and time to VS, we used fractional polynomial functions which provide a flexible way to model the shape of the relationship of a continuous variable with the outcome [23]. We used a set of defined powers (–2, –1, –0.5, 0.5, 1, 2 and ln(x)) and a maximum of two power terms in the model. The differences in model deviances were compared; the linear model was used if the improvement in fit was not statistically significant at

$p<0.05$. Mean VAS adherence during follow-up was calculated by taking the average adherence in the visits prior to achieving VS in those that suppressed or the average adherence in the visits prior to censoring in those that did not achieve VS. Missing adherence measurements were omitted. VAS adherence was transformed into a categorical variable using clinically meaningful cut-offs. VL was handled in a similar manner.

After fitting the full model, the proportional hazard assumption was tested both globally and for individual covariates, by regressing the scaled Schoenfeld residuals on time; the null hypothesis was that the slope was zero, i.e. that the log HR function was constant over time.

Results

Cohort description

Of the 1,557 participants who reported not to be on ART at entry, 1,328 (85.3%) had a VL>1,000 copies/mL of whom 1,148 (86.4%) had successful NGS of the HIV *pol* gene (consensus sequences available in GenBank, accession numbers: MH709380 – MH710527). Of the 1,148 with NGS data, 920 (80.1%) initiated ART within the trial of whom 837 individuals had at least one VL result after ART initiation (Figure 1).

Prevalence of any PDR or NNRTI DRM

Of the 1,148 participants who had their virus successfully sequenced, 109 (9.5%) had at least one PDR mutation detected at 20% threshold, NNRTI resistance being predominant with a prevalence of 101/1,148 (8.8%). The number of participants with any PDR mutation increased to 147 (12.8%) when minority variants were accounted for at 5% threshold (Figure 2). Prevalence of NRTI resistance was low with 12 (1.1%), and 23 (2.0%) participants out of 1,148 having NRTI DRM detected at 20% and 5% thresholds, while protease inhibitor resistance was found in 8 (0.7%) and 16 (1.4%) individuals respectively. Detailed description of the DRM are presented in Supplementary Figure 1 and Supplementary Figure 2.

Amongst those with resistance, dual class NRTI/NNRTI DRM were found in 6/109 (5.5%) and 11/147 (7.8%) participants with PDR at 20% and 5% thresholds respectively (Supplementary Table 3).

The majority of participants with virus sequences had a median age of 32.9 years (IQR 25.6-45.2), with characteristics described in Table 1. The median CD4 count at clinic presentation was 405cells/mm^3 (IQR 261-559) and the median VL was 4.5log10 copies/mL (IQR 3.9-5.2). There was no difference in the median age of individuals with sequences (n=1,148) and those without (n=409; 32.9 years (IQR 25.6-45.2) vs. 33.5 years (IQR 26.6-45.6), p=0.67]. A higher proportion of female than men had no virus sequences (28.1% vs. 21.4%, p=0.008).

Association of pre-treatment drug resistance with virologic suppression.

Of the 920 individuals who initiated ART (96.3% started Atripla®) and had virus sequence data, 837 had at least one follow-up VL and were used to examine the impact of PDR on response to therapy. There was no statistically significant difference in the completeness of VL measurements at each visit between individuals with and without PDR during the first 12 months of ART (Supplementary Table 4). Their median age was 34.3 years, 72% were female, and 83.5% had an overall mean VAS adherence ≥95% (Table 2). The 83 participants with missing VL data were younger than those with VL data [median age 29.5 years (IQR 23.5-41.6) vs. 34.3years (27.3-46.5); p=0.02] and a higher proportion were male (42% vs. 28%; p=0.009). The prevalence of any PDR at the 20% threshold in participants with and without VL data (9.4% vs 12.1%; p=0.44, respectively) was similar to that in all individuals with sequences (9.5%).

Amongst the 837 HIV-positive individuals who contributed to the analysis; 748 individuals had no PDR, 82 had NNRTI PDR only and 7 had both NRTI and NNRTI PDR at the 5% threshold. At the 20% threshold, the corresponding numbers were 765, 67 and 5 respectively. Participants were followed for a median of 1.36years (IQR 0.91-2.13) after ART initiation. At the 20% detection threshold, time to VS was longer for those with both NRTI/NNRTI PDR than those without any PDR [median 11.73months (IQR 2.76-16.39) vs. 3.45months (IQR 2.79-5.75)], whilst there was no significant difference between those with only NNRTI PDR compared to those with no PDR [median 4.11months (IQR 2.86-5.98) vs. 3.45months (IQR 2.79-5.75)] (Figure 3a) (log rank test overall; p=0.10) At the 5% detection threshold, time to VS was longer for those with both NRTI/NNRTI PDR than those without any PDR [median 11.73 months (IQR 2.76-16.39) vs. 3.48months (IQR 2.79-5.78)], whilst there was no difference between those with only NNRTI PDR compared to those with no PDR [median 3.71months (IQR 2.79-5.55) vs. 3.48months (IQR 2.79-5.78)] (Figure 3b) (log rank test overall; p=0.09) The median time to achieve VS, overall, was 3.61months (IQR 2.79-5.78). The overall cumulative probability of VS at 12 months was 94.5% (95% CI 92.7-96.0).

In unadjusted Cox models, for resistant variants detected at 20% (Table 3), there was an association between presence of both NRTI/NNRTI PDR with longer time to VS but this did not reach statistical significance (HR 0.42 (95% CI 0.16-1.12). However, there was no association with VS for those with only NNRTI PDR (HR 0.84 (0.64-1.11). Factors associated with longer time to VS were being male and having a high VL at baseline (>100,000 copies/mL), while a mean VAS adherence of ≥95% and a higher CD4 count at initiation were associated with shorter time to VS. In a multivariable Cox regression model that adjusted for age, sex, CD4 count and VL at ART initiation, and adherence, the association between having both NRTI/NNRTI PDR and VS remained virtually unchanged from the unadjusted model [adjusted (a)HR 0.41, 95%CI=0.15-1.10] with attenuation of the effect of association between having only NNRTI PDR and VS [aHR 0.90 (0.68-1.18)]. Having a high baseline VL was independently associated with significantly longer time to VS while VAS adherence ≥95% remained independently associated with shorter time to VS.

When we repeated the analysis taking into account the presence of resistant variants detected at the 5% threshold (Table 4), we found a statistically significant association between having both NRTI/NNRTI PDR and longer time to VS [both NRTI/NNRTI PDR vs. no PDR; aHR 0.32, 95%CI=0.12-0.86]. There was no difference in time to VS between having only NNRTI PDR and no PDR [aHR 1.05, 95%CI=0.82-1.34]

Discussion

We report the first study from the sub-Saharan HIV epidemic exploring NGS-defined DR, and response to currently recommended first-line FDC therapy. The prevalence of any PDR was 9.5% at 20% detection level, and up to 13% with a detection limit of 5% amongst HIV-positive individuals reporting no prior ART at entry into the trial. Virological response was similar between individuals who had only NNRTI PDR and those who had no PDR. However, VS was poorer in individuals who had dual-class NRTI/NNRTI PDR than in those without PDR at the 5% threshold. The association at the 20% threshold did not reach statistical significance most likely due to very small numbers of individuals with dual-class PDR.

Our findings contrast with two large cohort studies addressing a similar question in SSA, in which PDR defined by population sequencing was associated with virological failure or treatment switch when at least one drug was compromised in participants initiating first-line ART [4, 5]. The majority of participants in the cited studies were on AZT or d4T backbone in combination with either NVP or EFV. By contrast, only a third of the participants in these two studies were on TDF with either 3TC or FTC combined with NVP or EFV. Other similar studies in individuals prescribed predominantly older ART regimen have also shown an association between poorer virological response and PDR when at least one drug was compromised [6-8]. In our study with NGS-defined PDR, nearly all participants were on fixed dose combination TDF/FTC/EFV with VS being compromised only when PDR to at least two of the prescribed drugs was present. There was no difference in VS between patients with only NNRTI PDR and those with no PDR, a finding collaborated by a descriptive study which showed that virological response was similar in individuals with only NNRTI PDR and those with no PDR if on EFV-based ART, with poorer response observed only when both NRTI and NNRTI PDR were present [9].

Our findings suggest that the combination of TDF/FTC in the presence of good adherence is potent enough to achieve short-term VS despite the presence of NNRTI PDR. TDF/FTC/EFV was found to be either equivalent or superior to its comparator arms in a study comparing four WHO recommended regimens [24]. This observation was attributed to higher potency of EFV compared to NVP and the longer intracellular half of FTC-triphosphate [25] than 3TC-triphosphate [26] which could mean better forgiveness of FTC containing regimen with missed ART doses. These factors may explain our finding of little impact of only NNRTI PDR.

Some studies with small sample size have shown no association between PDR and virological outcomes [10-13].

Our PDR prevalence figures are similar to that of a recent study performed across all the South African provinces [27]. The high proportion of NNRTI resistance in this survey likely reflects the exposure of the population to NNRTI-based ART following the roll-out of the national HIV treatment programs. However, NRTI mutations, such as M184V which was present in our study, were unlikely to have been transmitted because of their fitness cost to the virus. Therefore, the presence of dual class NRTI/NNRTI mutations in our study may suggest previous ART exposure in patients who did not report it, as suggested in previous studies [27, 28]. Moreover, the use of NGS to detect minority variants at ART initiation could be clinically relevant, as poorer VS was observed in participants with NRTI/NNRTI detected at the 5% threshold.

Our study has a few limitations. About 15% of participants had VL<1,000 copies/mL at entry, hence did not have virus sequenced. If this was due to undisclosed prior ART, we could have underestimated the prevalence of PDR in the population of HIV-positive individuals initiating or re-initiating ART. More females did not have sequences either because of low plasma VL or failure of sequencing. However, as there was no difference in the prevalence of PDR between males and females amongst those sequenced, we do not believe this would have biased our estimates of PDR. A small proportion (9.0%) of individuals with missing follow-up VL could not be evaluated for virological response. These were younger and more likely to be male; characteristics associated with poorer VS in our cohort [29], hence we could have overestimated virological response in the studied sample. However, this is unlikely due to the small number of participants with missing VL.

The NNRTI DR threshold for considering a change in the first-line ART in a public health approach in low- and middle-income countries was recently lowered from 15% to 10% by the WHO [30, 31], with dolutegravir (DTG)-based first-line ART poised to replace EFV [32, 33] because of its higher VS rates, shorter time to VS and fewer side-effects [34, 35]. The precise impact of NRTI PDR on response to tenofovir/lamivudine/dolutegravir remains to be seen, although NNRTI PDR alone will not compromise this regimen. Moreover, there are also limited data on the use of DTG in patients with tuberculosis [36] which is prevalent in SSA, and in pregnancy [37] with recent data from Botswana suggesting a higher frequency of neural tube birth defects in women who conceived on DTG [38]. Hence there would still be HIV-positive individuals whom an EFV-based ART may be more appropriate.

In conclusion, in the setting of a community trial involving a large study population initiating a FDC of TDF/FTC/EFV in HIV-positive individuals, we found no association between the presence of only NNRTI PDR and VS, however PDR to both NRTI and NNRTI was associated with longer time to VS. Good ART adherence and the high potency of TDF/FTC/EFV may have compensated for the presence of only NNRTI PDR. Studies with longer duration of follow-up in real life public ART programmes are warranted to properly quantify the effect of PDR on clinical outcomes in the African setting, as new first-line regimens are rolled out.

NOTES

Author contributions: CI, AD, DP and FD designed and implemented the study. AD generated and analyzed the sequencing data. CI, AD, KB did the statistical analyses. CI, AD and DP wrote the initial draft of the manuscript. All authors contributed to the interpretation and presentation of the findings. All authors approved the final version of the manuscript for submission.

Acknowledgements: We thank Doctor Jennifer Giandhari, Shyamala Padayachi, Zizile Sikhosana and Sureshnee Pillay for their assistance with sample processing and sequencing. Special thanks to Professor Jean-François Delfraissy, Director of ANRS. We thank the study volunteers for allowing us into their homes and participating in this trial and the Department of Health of South Africa for their support of this study.

Disclaimer. The content is solely the responsibility of the authors and does not represent the official views of 3ie or the Bill & Melinda Gates Foundation. The funders had no role in the design, analysis, and interpretation of the study or the decision to submit for publication.

Funding: The TasP trial was supported by the French National Agency for Aids and Viral Hepatitis Research (ANRS) [grant number ANRS 2011-375]; the Deutsche Gesellschaft für Internationale Zusammenarbeit [grant number 81151938]; the International Initiative for Impact Evaluation, Inc (3ie), with support from the Bill & Melinda Gates Foundation. We acknowledge BMGF for also supporting the PANGEA_HIV consortium (PI Pillay). Collins Iwuji also received additional funding from the People Programme (Marie Curie Actions) of the European Union's seventh Framework Programme FP7/2007-2013 [grant number 612216]. Tulio de Oliveira research is funded by the South African Medical Research Council (grant number MRC-RFA-UFSP-01-2013/UKZN HIVEPI) and by the Royal Society Newton Advanced Fellowship. The trial is conducted with the support of Merck & Co. Inc and Gilead Sciences that provided the Atripla® drug supply. The Africa Health Research Institute receives core funding from the Wellcome Trust, which provides the platform for the population- and clinic-based research at the Centre.

Potential conflicts of interests. CI received honoraria for consulting services from Gilead Sciences. Dr. Marcelin reports grants and personal fees from VIIV Healtcare, grants and personal fees from Gilead, grants and personal fees from MSD, grants and personal fees from Janssen, outside the submitted work. All other authors declare no potential conflicts

References

1. WHO. Global AIDS Update 2016. Available at: http://www.who.int/hiv/pub/arv/global-AIDS-update-2016_en.pdf?ua=1.

2. Lessells RJ, Mutevedzi PC, Iwuji CC, Newell ML. Reduction in early mortality on antiretroviral therapy for adults in rural South Africa since change in CD4+ cell count eligibility criteria. J Acquir Immune Defic Syndr **2014**; 65(1): e17-24.

3. Tanser F, Bärnighausen T, Grapsa E, Zaidi J, Newell ML. High coverage of ART associated with decline in risk of HIV acquisition in rural KwaZulu-Natal, South Africa. Science **2013**; 339(6122): 966-71.

4. Boender TS, Hoenderboom BM, Sigaloff KC, *et al*. Pretreatment HIV drug resistance increases regimen switches in sub-Saharan Africa. Clin Infect Dis **2015**; 61(11): 1749-58.

5. Hamers RL, Schuurman R, Sigaloff KC, et al. Effect of pretreatment HIV-1 drug resistance on immunological, virological, and drug-resistance outcomes of first-line antiretroviral treatment in sub-Saharan Africa: a multicentre cohort study. Lancet Infect Dis **2012**; 12(4): 307-17.

6. Chung MH, Beck IA, Dross S, *et al*. Oligonucleotide ligation assay detects HIV drug resistance associated with virologic failure among antiretroviral-naive adults in Kenya. J Acquir Immune Defic Syndr **2014**; 67(3): 246-53.

7. Hong SY, Jonas A, DeKlerk M, et al. Population-based surveillance of HIV drug resistance emerging on treatment and associated factors at sentinel antiretroviral therapy sites in Namibia. J Acquir Immune Defic Syndr **2015**; 68(4): 463-71.

8. Kantor R, Smeaton L, Vardhanabhuti S, et al. Pretreatment HIV Drug Resistance and HIV-1 Subtype C Are Independently Associated With Virologic Failure: Results From the Multinational PEARLS (ACTG A5175) Clinical Trial. Clin Infect Dis **2015**; 60(10): 1541-9.

9. Beck I, Levine M, Milne R, et al. Impact of Pre-Treatment HIV-Drug Resistance on Virologic Outcome of First-Line NNRTI-ART. CROI 2017. Seattle, USA.

10. Lee GQ, Bangsberg DR, Muzoora C, *et al.* Prevalence and virologic consequences of transmitted HIV-1 drug resistance in Uganda. AIDS Res Hum Retroviruses **2014**; 30(9): 896-906.

11. Rusine J, Asiimwe-Kateera B, van de Wijgert J, *et al*. Low primary and secondary HIV drug-resistance after 12 months of antiretroviral therapy in human immune-deficiency virus type 1 (HIV-1)-infected individuals from Kigali, Rwanda. PLoS One **2013**; 8(8): e64345.

12. Mzingwane ML, Tiemessen CT, Richter KL, Mayaphi SH, Hunt G, Bowyer SM. Pre-treatment minority HIV-1 drug resistance mutations and long term virological outcomes: is prediction possible? Virol J **2016**; 13(1): 170.

13. Zoufaly A, Jochum J, Hammerl R, *et al*. Virological failure after 1 year of first-line ART is not associated with HIV minority drug resistance in rural Cameroon. J Antimicrob Chemother **2015**; 70(3): 922-5.

14. Iwuji CC, Orne-Gliemann J, Larmarange J, *et al*. ANRS 12249 TasP Study Group. Universal test and treat and the HIV epidemic in rural South Africa: a phase 4, open-label, community cluster randomised trial. Lancet HIV **2017**; S2352-3018(17): 30205-9.

15. Massyn N, Peer N, English R, Padarath A, Barron P, Day C. District Health Barometer 2015/2016: Health Systems Trust, **2016**.

16. Iwuji CC, Orne-Gliemann J, Tanser F, *et al*. ANRS 12249 TasP Study Group. Evaluation of the impact of immediate versus WHO recommendations-guided antiretroviral therapy initiation on HIV incidence: the ANRS 12249 TasP (Treatment as Prevention) trial in Hlabisa sub-district, KwaZulu-Natal, South Africa: study protocol for a cluster randomised controlled trial. Trials **2013**; 14: 230.

17. Iwuji CC, Orne-Gliemann J, Larmarange J, *et al*. ANRS 12249 TasP trial group. Uptake of Home-Based HIV Testing, Linkage to Care, and Community Attitudes about ART in Rural KwaZulu-Natal, South Africa: Descriptive Results from the First Phase of the ANRS 12249 TasP Cluster-Randomised Trial. PLoS Med **2016**; 13(8): e1002107.

18. Oyugi JH, Byakika-Tusiime J, Charlebois ED, *et al*. Multiple validated measures of adherence indicate high levels of adherence to generic HIV antiretroviral therapy in a resource-limited setting. J Acquir Immune Defic Syndr **2004**; 36(5): 1100-2.

19. Gall A, Ferns B, Morris C, *et al*. Universal amplification, next-generation sequencing, and assembly of HIV-1 genomes. J Clin Microbiol **2012**; 50(12): 3838-44.

20. Kearse M, Moir R, Wilson A, *et al*. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics **2012**; 28(12): 1647-9.

21. Lapointe HR, Dong W, Lee GQ, *et al*. HIV drug resistance testing by high-multiplex "wide" sequencing on the MiSeq instrument. Antimicrob Agents Chemother **2015**; 59(11): 6824-33.

22. Bennett DE, Camacho RJ, Otelea D, *et al*. Drug resistance mutations for surveillance of transmitted HIV-1 drug-resistance: 2009 update. PLoS One **2009**; 4(3): e4724.

23. Royston P, Ambler G, Sauerbrei W. The use of fractional polynomials to model continuous risk variables in epidemiology. Int J Epidemiol **1999**; 28(5): 964-74.

24. Tang MW, Kanki PJ, Shafer RW. A review of the virological efficacy of the 4 World Health Organization-recommended tenofovir-containing regimens for initial HIV therapy. Clin Infect Dis **2012**; 54(6): 862-75.

25. Wang LH, Begley J, St Claire RL, 3rd, Harris J, Wakeford C, Rousseau FS. Pharmacokinetic and pharmacodynamic characteristics of emtricitabine support its once daily dosing for the treatment of HIV infection. AIDS Res Hum Retroviruses **2004**; 20(11): 1173-82.

26. Yuen GJ, Lou Y, Bumgarner NF, et al. Equivalent steady-state pharmacokinetics of lamivudine in plasma and lamivudine triphosphate within cells following administration of lamivudine at 300 milligrams once daily and 150 milligrams twice daily. Antimicrob Agents Chemother **2004**; 48(1): 176-82.

27. Steegen K, Carmona S, Bronze M, *et al*. Moderate Levels of Pre-Treatment HIV-1 Antiretroviral Drug Resistance Detected in the First South African National Survey. PLoS One **2016**; 11(12): e0166305.

28. Gupta R, Gregson J, Parkin N, Haile-Selassie H, Tanuri A, Andrade Forero L, Kaleebu P, Watera C, Aghokeng A, Mutenda N, Dzangare J, Hone S, Hang ZZ, Garcia J, Garcia Z, Marchorro P, Beteta E, Giron A, Hamers R, Inzaule S, Frenkel LM, Chung MH, de Oliveira T, Pillay D, Naidoo K, Kharsany A, Kugathasan R, Cutino T, Hunt G, Avila Rios S, Doherty M, Jordan MR, Bertagnolio S. HIV-1 drug resistance before initiation or re-initiation of first-line antiretroviral therapy in low-income and middle-income countries: a systematic review and meta-regression analysis. Lancet Infect Dis **2018**; 18(3): 346-55.

29. Iwuji C, Pillay S, Derache A, Danaviah S, Baisley K, de Oliveira T, Dabis F, Porter K, Pillay D for the ANRS 12249 TasP Study Group. Virologic suppression and emerging resistance on first-line antiretroviral therapy following universal test and treat: the ANRS 12249 cluster randomised trial. 9th IAS Conference on HIV Science. Paris, France, **2017**.

30. Phillips AN, Stover J, Cambiano V, *et al*. Impact of HIV Drug Resistance on HIV/AIDS-Associated Mortality, New Infections, and Antiretroviral Therapy Program Costs in Sub-Saharan Africa. J Infect Dis **2017**; 215(9): 1362-5.

31. WHO. Guidelines on the public health response to pretreatment HIV drug resistance, **2016**.

32. UNAIDS. New high-quality antiretroviral therapy to be launched in South Africa, Kenya and over 90 low-and middle-income countries at reduced price. **2017**.

33. Venter WF, Kaiser B, Pillay Y, *et al*. Cutting the cost of South African antiretroviral therapy using newer, safer drugs. S Afr Med J **2016**; 107(1): 28-30.

34. Walmsley S, Baumgarten A, Berenguer J, *et al*. Dolutegravir Plus Abacavir/Lamivudine for the Treatment of HIV-1 Infection in Antiretroviral Therapy-Naive Patients: Week 96 and Week 144 Results From the SINGLE Randomized Clinical Trial. J Acquir Immune Defic Syndr **2015**; 70(5): 515-9.

35. Walmsley SL, Antela A, Clumeck N, *et al*. SINGLE Investigators. Dolutegravir plus abacavir-lamivudine for the treatment of HIV-1 infection. N Engl J Med **2013**; 369(19): 1807-18.

36. Cevik M, Vincent R, McGann H. Use of dolutegravir in combination with rifampicin based TB therapy in HIV/TB co-infected patients. Glasgow HIV 2016, **2016**.

37. Kandel CE, Walmsley SL. Dolutegravir - a review of the pharmacology, efficacy, and safety in the treatment of HIV. Drug Des Devel Ther **2015**; 9: 3547-55.

38. WHO.
Potential safety issue affecting women living with HIV using dolutegravir at the time of conception Available at: http://www.who.int/medicines/publications/drugalerts/Statement_on_DTG_18May_2018final.pdf?ua=1. Accessed 05 Jun 2018.

Table 1. Demographic and clinical characteristics of all participants assessed for pre-treatment drug resistance*.

Characteristics of individuals with sequences	**Total N=1,148 (%)**	**Individuals without pre-treatment HIV drug resistance n=1, 039(%)**	**Individuals with pre-treatment HIV drug resistance n=109 (%)**
Age (Years)			
Median age (IQR)	32.9 (25.6-45.2)	33.3 (25.8-45.8)	30.0 (25.0-36.4)
16-29	463 (40.3)	409(39.4)	54 (49.5)
30-39	298 (26.0)	267 (25.7)	31 (28.4)
40-49	178 (15.5)	168 (16.2)	10 (9.2)
>50	202 (17.6)	189 (18.2)	13 (11.9)
Missing	7 (0.6)	6 (0.6)	1 (0.9)
Sex			
Female	807 (70.3)	729 (70.2)	78 (71.6)
Male	341 (29.7)	310 (29.8)	31 (28.4)
CD4 at presentation *			
Median (IQR) cells/mm^3	404 (261-559)	405 (261-559)	383 (263-533)
<350	448 (39.0)	404 (38.9)	44 (40.4)
350-500	299 (26.1)	270 (26.0)	29 (26.6)
>500	379 (33.0)	348 (33.5)	31 (28.4)
Missing	22 (1.9)	17 (1.6)	5 (4.6)

Characteristics of individuals with sequences	Total N=1,148 (%)	Individuals without pre-treatment HIV drug resistance n=1, 039(%)	Individuals with pre-treatment HIV drug resistance n=109 (%)
Viral load* copies/mL			
Median (Log10)	4.5 (3.9-5.2)	4.5 (3.9-5.2)	4.6 (4.1-5.1)
<10,000	309 (26.9)	285 (27.4)	24 (22.0)
10,000-100,000	478 (41.6)	429 (41.3)	49 (45.0)
>100,000	356 (31.0)	320 (30.8)	36 (33.0)
Missing	5 (0.4)	5 (0.5)	0 (0.0)
Education			
Primary or less	483 (42.1)	432 (41.6)	51 (42.5)
Some secondary	427 (37.2)	385 (37.1)	47 (39.2)
Secondary or higher	234 (20.4)	218 (21.0)	22 (18.3)
Missing	4 (0.4)	4 (0.3)	0 (0.0)
Marital status			
Never married	1,009 (87.9)	904 (87.0)	105 (96.3)
Married	92 (8.0)	89 (8.6)	3 (2.8)
Divorced/Separated	43 (3.8)	42 (4.0)	1 (0.9)
Missing	4 (0.4)	4 (0.4)	0 (0.0)
Employment			

Characteristics of individuals with sequences	Total N=1,148 (%)	Individuals without pre-treatment HIV drug resistance n=1, 039(%)	Individuals with pre-treatment HIV drug resistance n=109 (%)
Employed	166 (14.5)	155 (14.9)	11 (10.1)
Student	60 (5.2)	53 (5.1)	7 (6.4)
Unemployed	917 (79.9)	826 (79.5)	91 (83.5)
Missing	5 (0.4)	5 (0.5)	0 (0·0)
Receiving government grants			
Yes	662 (57.7)	597 (57.5)	65 (59.6)
No	473 (41.2)	429 (41.3)	44 (40.4)
Missing	13 (1.1)	13 (1.3)	0 (0.0)

*Pre-treatment drug resistance is defined by NGS only

Table 2: Baseline characteristics of individuals contributing to the analysis of virological suppression.

	In analysis n=837 (%)	Missing VL N=83(%)	P value
Age at initiation (Years)			
Median age (IQR)	34.3 (27.3, 46.5)	29.5 (23.5, 41.6)	0.02
16-29	290 (34.6)	43 (51.8)	
30-39	246 (29.4)	15 (18.1)	
40-49	133 (15.9)	9 (10.8)	
>50	166 (19.8)	13 (15.7)	
Missing	2 (0.2)	3 (3.6)	
Sex			
Female	599 (71.6)	48 (57.8)	0.009
Male	238 (28.4)	35 (42.2)	
CD4 at initiation			
Median (IQR) cells/mm^3	348 (227, 480)	399 (235, 521)	0.630
<=350	418 (49.9)	37 (44.6)	
350-500	230 (27.5)	20 (24.1)	
>500	182 (21.7)	22 (26.5)	
Missing	7 (0.8)	4 (4.8)	
Viral load copies/mL			
Median (Log copies/mL)	4.6 (4.0, 5.2)	4.6 (3.9, 5.2)	0.818

<10000	200 (23.9)	22 (26.5)	
10000-100000	350 (41.8)	36 (43.3)	
>100000	285 (34.1)	25 (30.1)	
Missing	2 (0.2)	0 (0.0)	
Adherence (%)			
<95	126 (15.1)	-	
≥95	699 (83.5)	-	
Missing	12 (1.4)	-	
ART regimen			0.001
TDF+FTC+EFV	806 (96.3)	73 (88.0)	
TDF+3TC+EFV	6 (0.7)	2 (2.4)	
AZT+3TC+ EFV	18 (2.2)	3 (3.6)	
D4T+3TC+EFV	1 (0.1)	-	
AZT+3TC+PI	1 (0.1)	-	
Missing	5 (0.6)	5 (6.0)	

TDF=tenofovir, FTC=emtricitabine, EFV=efavirenz, 3TC=lamivudine, AZT=zidovudine, D4T=stavudine, IQR interquartile range

Table 3. Factors associated with virologic suppression in adults with PDR detected at the 20% threshold

Characteristics	Unadjusted hazard ratio (95% CI)	P value	Adjusted hazard ratio (95% CI)	P value
Pretreatment Drug Resistance		0.06		0.09
No PDR	1		1	
Only NNRTI PDR	0.84 (0.64-1.11)		0.90 (0.68-1.18)	
Both NNRTI/NRTI PDR	0.42 (0.16-1.12)		0.41 (0.15-1.10)	
Age at initiation/5 years	1.02 (1.00-1.05)	0.11	1.03 (1.00-1.06)	0.06
Sex		0.01		0.69
Female	1		1	
Male	0.82 (0.70-0.96)		0.97 (0.82-1.14)	
CD4 at initiation/100 cells/mm^3	1.06 (1.03-1.09)	<0.001	1.03 (1.00-1.06)	0.10
Viral load copies/mL		<0.001		<0.001
≤10000	1		1	
10000-100000	0.74 (0.61-0.88)		0.75 (0.62-0.90)	
>100000	0.47 (0.38-0.56)		0.48 (0.39-0.59)	
VAS Adherence (%)		0.001		0.003
<95	1		1	
≥95	1.40 (1.14-1.73)		1.37 (1.11-1.70)	

Table 4. Factors associated with virologic suppression in adults with PDR detected at the 5% threshold

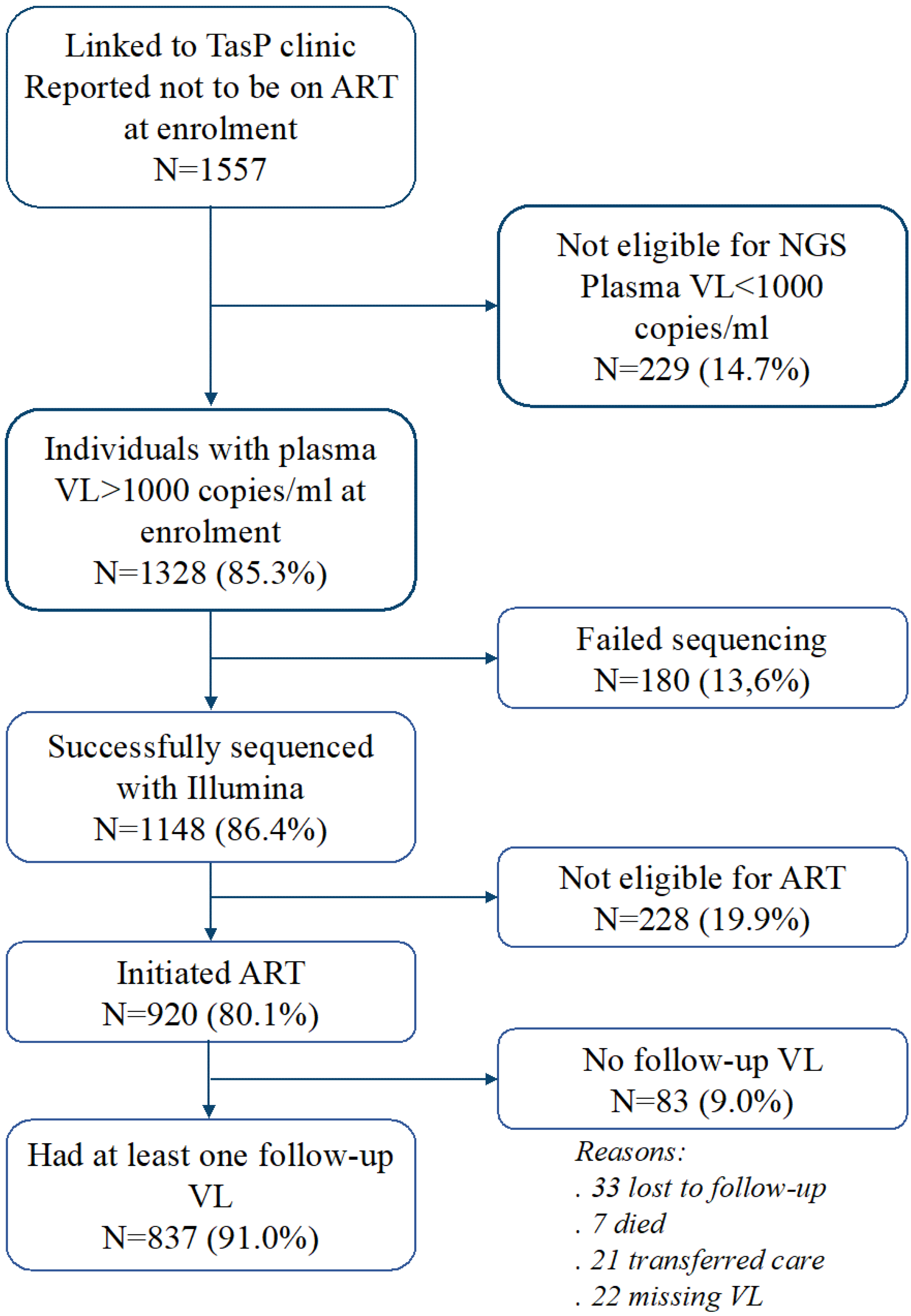
Characteristics	Unadjusted hazard ratio (95% CI)	P value	Adjusted hazard ratio (95% CI)	P value
Pretreatment Drug Resistance				0.02
No PDR			1	
Only NNRTI PDR			1.05 (0.82-1.34)	
Both NNRTI/NRTI PDR			0.32 (0.12-0.86)	
Age at initiation/5 years	1.02 (1.00-1.05)	0.11	1.03 (1.00-1.06)	0.05
Sex		0.01		0.70
Female	1		1	
Male	0.82 (0.70-0.96)		0.97 (0.82-1.14)	
CD4 at initiation/100 cells/mm^3	1.06 (1.03-1.09)	<0.001	1.03 (1.00-1.06)	0.09
Viral load copies/mL		<0.001		<0.001
≤10000	1		1	
10000-100000	0.74 (0.61-0.88)		0.74 (0.61-0.89)	
>100000	0.47 (0.38-0.56)		0.47 (0.39-0.58)	
VAS Adherence (%)		0.001		0.003
<95	1		1	
≥95	1.41 (1.14-1.73)		1.38 (1.11-1.70)	

Figure 1. Cohort flow chart

Figure 2. Prevalence of any pretreatment drug resistance and NNRTI resistance among 1,148 participants with NGS data detected at 5% and 20% detection thresholds.

Figure 3. Kaplan Meier plot of the cumulative probability of VS since ART start: stratified by class of PDR at the 20% (3a) and 5% (3b) detection thresholds.

Figure 1



Linked to TasP clinic
Reported not to be on ART at enrolment
N=1557
Not eligible for NGS
Plasma VL<1000 copies/ml
N=229 (14.7%)
Individuals with plasma VL>1000 copies/ml at enrolment
N=1328 (85.3%)
Failed sequencing
N=180 (13,6%)
Successfully sequenced with Illumina
N=1148 (86.4%)
Not eligible for ART
N=228 (19.9%)
Initiated ART
N=920 (80.1%)
No follow-up VL
N=83 (9.0%)
Reasons:
. 33 lost to follow-up
. 7 died
. 21 transferred care
. 22 missing VL
Had at least one follow-up VL
N=837 (91.0%)

Figure 2

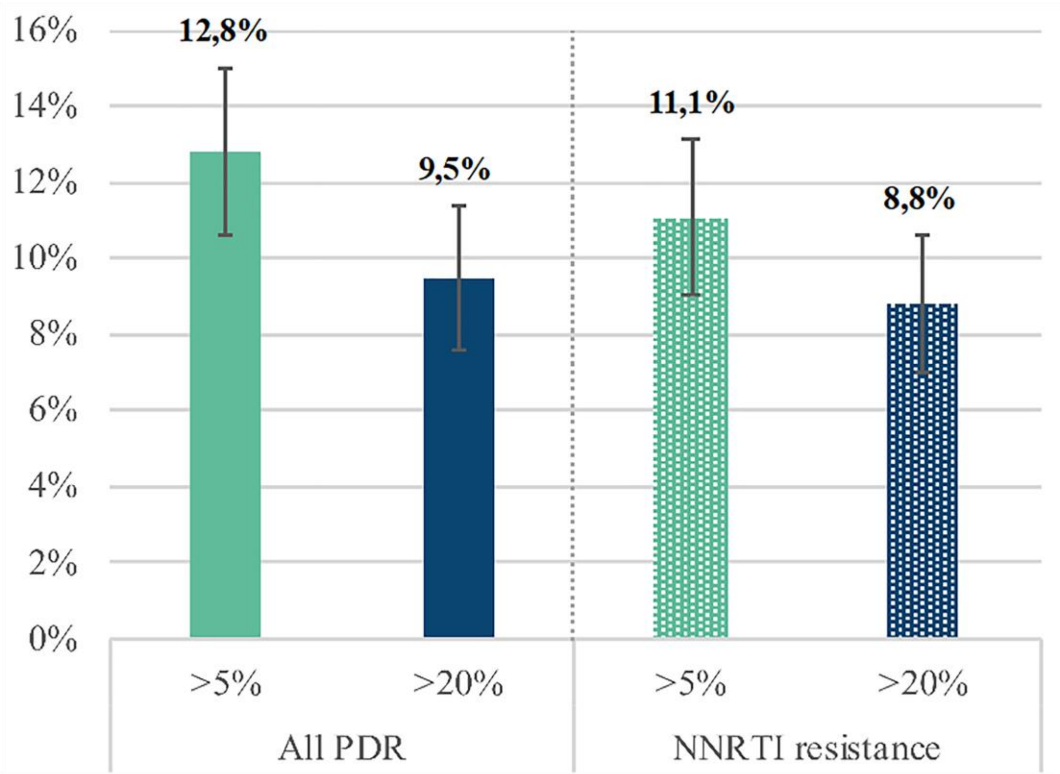
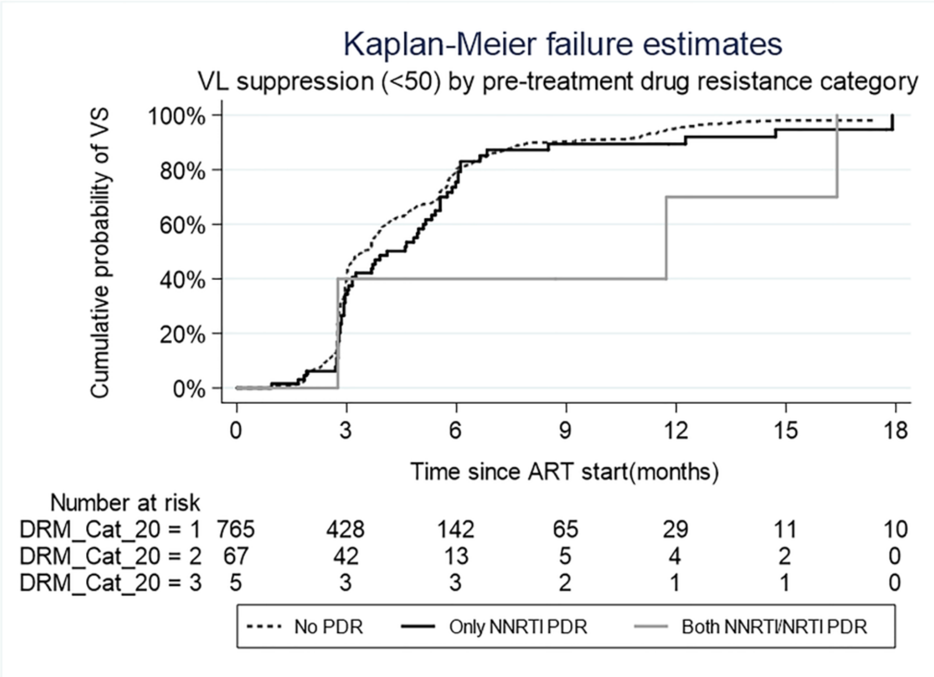
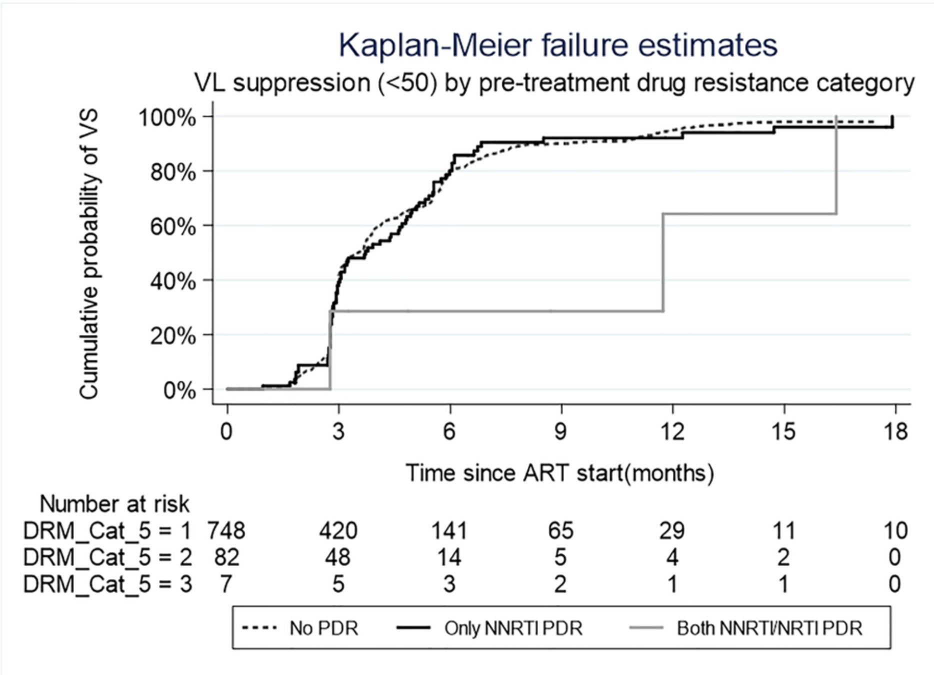

16%
14%
12%
10%
8%
6%
4%
2%
0%
12,8%
9,5%
11,1%
8,8%
>5%
>20%
>5%
>20%
All PDR
NNRTI resistance

Figure 3



3a



3b