

Implementation of an intensive adherence intervention in patients with second-line ART failure in four West African countries with limited access to genotypic resistance testing: a prospective cohort study

Revised version 2

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Abstract (300 words)

Background: Switching to third-line ART in patients who fail second-line is a difficult decision in settings with limited access to genotypic resistance testing (GRT). We used a standardized algorithm including a wide range of adherence-enhancing interventions followed by a new viral load (VL) measurement to decide whether to switch to third-line. The decision, based on the effectiveness of the adherence reinforcement to drive viral resuppression, did not use GRT.

Methods: Between March 2013 and August 2016, adults failing a PI-boosted-based second-line ART in four West African countries were proposed 9 adherence reinforcement interventions, and followed 64 weeks. We performed a new VL at week-12 and used its results for a decision at week-16: In case of successful resuppression, patients continued the same second-line regimen; Otherwise, they switched to a darunavir/r-altegravir-based third-line. The primary endpoint was virologic success at week-64. After study termination we performed GRT on frozen plasma samples collected at baseline. Week-16 decision appropriateness was retrospectively judged based on the baseline Genotypic Sensitivity Score (GSS).

Findings: Of the 198 participants, 5 died before week-16. Of the 193 remaining, 130 (66%) reached viral resuppression and continued with second-line and 63 (33%) switched to third-line at week-16. Post-study GRT showed that the baseline GSS was <2 in 34% of participants, and led to retrospectively conclude that the week-16 decision blinded to resistance data was appropriate in 145 (75%) patients. At week-64, 4 patients (2%) were lost to follow-up, 10 (5%) were dead and 101 (52%) had a VL <50 copies per mL.

Interpretation: Poor adherence is the first problem to tackle in patients for whom second-line ART is failing when resistance tests are not routinely available and is effectively a manageable problem. Lack of access to GRT should not be an obstacle to the prescription of third-line ART in patients who do not achieve viral resuppression after adherence reinforcement.

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Research in context

Evidence before this study

We searched PubMed for publications before December 2018 with the terms (HIV AND antiretroviral therapy AND sub-Saharan Africa AND (third-line OR second-line OR failure)), without any language or date restrictions. We found descriptions of HIV drug resistance mutations among patients failing second-line antiretroviral therapy; evidence that poor adherence to PI-based therapy does not consistently lead to resistance patterns that require the use of third-line drugs; and non-randomized evidence that reinforcing adherence in patients failing first or second-line ART allowed a great percentage of patients to achieve effective viral resuppression and therefore continue their initial therapy. We did not find any prospective study of third-line ART in sub-Saharan Africa.

Added value of this study

Our study provides for the first time prospective data on the sequence between second-line failure and third-line efficacy in West Africa, in a context where the decision to move from second to third line is taken on the basis of the return or not to viral suppression after reinforcement of the adherence. Our results also provide the first prospective data on the efficacy of darunavir plus raltegravir-based third-line therapy in West Africa. Because we performed resistance tests after the end of the study, we were able to get a comprehensive picture of the resistance profile of patients who achieved or not viral resuppression after adherence reinforcement, and to estimate the risk of resistance accumulation when continuing second-line on the basis of viral resuppression. The adherence reinforcement-based algorithm allowed appropriate decisions to be made on whether or not to switch to third-line in 75% of patients. Repeating the viral load every three months further decreased the percentage of patients inappropriately maintained on second-line.

Implications of all the available evidence

In settings where genotype resistance tests are not available, an algorithm based on intensive adherence reinforcement and repeated viral load monitoring is a reasonable strategy for deciding whether to switch to third-line ART in patients on second-line ART with virological failure. Poor adherence is the first problem to tackle in patients for whom second-line ART is failing and is effectively a manageable problem. Lack of access to resistance tests should no longer be an obstacle to the prescription of third-line ART.

Introduction

The UNAIDS 2018 report indicates that 21.7 million HIV-infected people receive antiretroviral therapies (ART) worldwide, 70% of them in sub-Saharan Africa. Only 10% of the latter receive second-line or third-line ART **(1)**, a proportion far below the proportion of patients who fail first-line and second-line ART in low and middle income countries **(2)**.

There are two main reasons for the low proportion of patients receiving second and third-line ART in sub-Saharan Africa: the difficulty in diagnosing ART failure due to low accessibility of viral load monitoring, and insufficient training of physicians in managing ART failure **(3)**. Two additional factors specifically hinder the use of third-line drugs: low availability of third-line drugs, which means it is all the more crucial to reserve them for people who really need them; and low access to genotypic resistance testing (GRT), which is needed more critically when switching from second-line to third-line compared to switching from first-line to second-line; and **(4,5)**.

The first cause of ART failure is poor adherence, which leads to drug resistance through suboptimal viral suppression. Total non-adherence to ART is associated with low probabilities of resistance while intermediate levels of adherence increase the risk of resistance **(6)**. In patients on second-line ART with virological failure, it is important to differentiate between patients whose virus is still sensitive to second-line drugs, and those bearing a resistant virus. The former may benefit from maintaining a second-line regimen but require effective interventions to strengthen adherence. The latter need to switch to a third-line regimen rapidly as delaying the decision to switch in patients with suboptimal viral suppression may lead to the accumulation of resistance mutations **(7)**.

In contexts where resistance testing is not widely available, the application of energetic adherence reinforcement interventions has been proposed for patients who fail second-line ART, followed by performance of a second viral load measurement, to differentiate patients who should continue with second-line from those who really need third-line drugs **(8,9)**. However, the efficacy of interventions to strengthen adherence may vary by context **(10)**.

In this study conducted in HIV-infected adults failing second-line ART in West Africa, we used a standardized algorithm including a wide range of adherence-enhancing interventions followed by a new viral load measurement at three months to decide whether to switch to third-line. The decision was based on the effectiveness of the adherence enhancement to drive viral resuppression and did not use resistance testing. After the end of the study, genotypic resistance

tests were performed on plasma samples taken and frozen at inclusion, and the adequacy of the decision to switch or not to third-line was retrospectively appraised on the basis of the resistance profile at baseline.

Methods

Study design and participants

Thilao was a cohort study recruiting adults failing a second-line ART in four West African countries. Participants were proposed adherence reinforcement interventions, and followed 64 weeks. Figure 1 summarizes the study design.

Thilao was conducted between March 2013 and August 2016 in Bobo-Dioulasso and Ouagadougou (Burkina Faso), Abidjan (Côte d'Ivoire), Bamako (Mali), and Dakar (Senegal). In the four countries: First-line regimens comprise tenofovir or zidovudine, plus lamivudine or emtricitabine, plus efavirenz or nevirapine; Second-lines include lamivudine or emtricitabine, plus tenofovir or zidovudine or abacabir, plus ritonavir-boosted atazanavir (ATV/r) or ritonavir-boosted lopinavir (LPV/r); And third-line consists of ritonavir-boosted darunavir (DRV/r) plus raltegravir, plus an optimized Nucleoside Reverse Transcriptase Inhibitor (NRTI) backbone. The latter is ideally guided by resistance tests, although in routine practice access to resistance testing is rare.

Patients were eligible in the study if they met all the following criteria: age ≥ 18 years, documented HIV-1 infection, history of failing an Non-Nucleoside Reverse Transcriptase Inhibitor (NNRTI)-based first-line ART, ongoing ATV/r or LPV/r based second-line ART > 6 months, confirmed plasma HIV-1 RNA > 1000 copies per mL (last measurement < 1 month), and a signed informed consent. Pregnant and breastfeeding women, HIV-2 infected or HIV-1+2 dually-infected patients, patients with severe renal or hepatic failure, and those with severe ongoing AIDS-related illnesses were excluded.

The study protocol was approved by the national ethics committees of the four countries.

Procedures

There were two phases to the study: Inclusion to week-16, and week-16 to week-64.

From inclusion to week-16, participants continued their previous second-line therapy while following an intensive adherence reinforcement program. The program consisted of monthly therapeutic education sessions, during which they chose as many adherence reinforcement interventions as they wished from the following nine options: (i) Direct observation of treatment

(DOT) by a relative (involving someone from their immediate circle to help them adhere to ART); (ii) Use of a pill organizer; (iii) Weekly phone calls (choosing one day of the week for the therapist to phone them to discuss treatment adherence); (iv) Daily alarm reminders (programming alarms on their phone at times scheduled for drug intake); (v) Daily SMS texts (receiving text messages that include symbols representing the tablets at times scheduled for drug intake); (vi) Home visits (receiving visits at home to discuss in a family environment the possible issues that may hinder compliance); (vii) Individual adherence reinforcement sessions (attending extra visits to the study center for individual adherence reinforcement sessions with trained health workers); (viii) Support group (participating in a self-help group to share experiences with peers); and (ix) Limitation of non-ART drugs prescribed concomitantly to ART (rationalizing prescriptions leading to remove nonessential medicines)(10). Explanation of how these interventions were chosen are given in the Supplemental Appendix, page 2. A new plasma HIV-1 RNA measurement was performed at week-12. The result was available to the physician four weeks later. This was used at week-16 to make a decision regarding the ART regimen: if plasma HIV-1 RNA was below 400 copies per mL or had decreased by more than two log₁₀ copies per mL compared to the baseline value, patients were asked to continue with second-line treatment; otherwise, they were asked to switch to a third-line therapy with DRV/r 600/100 mg BID (Prezista®, Janssen) plus raltegravir 400 mg BID (Isentress®, Merck Sharp Dohme), plus two recycled NRTIs.

From week-16 to week-64, patients had scheduled visits every four weeks. All patients, including those who stayed on second-line and those who switched to third-line, maintained the ability to choose or change every month the same adherence reinforcement interventions, as during the first phase, for the entire study follow-up. Plasma HIV-1 RNA was measured every 12 weeks. Patients with detectable plasma HIV-1 RNA at any time during follow-up had an additional new measurement four weeks later. Patients who stayed on second-line at week-16 were switched to third-line whenever they had two consecutive viral loads above 100 copies per mL.

After the end of the study, genotypic resistance tests were performed: in baseline plasma sample taken on inclusion and frozen at -80°C for all participants; and in week-64 samples for participants with a detectable viral load at week-64. The sequencing procedure and resistance interpretation used the ANRS 2018 algorithm (www.hivfrenchresistance.org). A genotypic susceptibility score (GSS) was calculated by translating the interpretations 'susceptible', 'intermediate resistance' and 'resistance' into scores of 1, 0.5 and 0, respectively. The GSS was

the sum of the individual scores for each of the drugs comprising the ART regimen that the patient was on at the time the plasma sample was collected. Ritonavir was not included in the score as a single drug.

Finally, plasma concentrations of antiretroviral drugs were measured in all patients with a VL>50 copies per mL at week-64, on the same plasma sample. Concentrations were considered inadequate when the minimal plasma concentrations were below the respective IC₅₀₋₉₀ of antiretroviral drugs, or below the respective limits of quantification (5 ng per mL for darunavir, raltegravir and tenofovir; 10 ng per mL for abacavir, lamivudine, didanosine, lopinavir and ritonavir)(11).

Outcomes

The main outcome of the study was successful virological suppression (plasma HIV-1 RNA <50 copies per mL) at week-64. Secondary outcomes were: severe adverse events; evolution of CD4 cell count; and appropriateness of the decision taken at week-16. When the baseline GSS was entirely calculable (amplifiable PR and RT sequences), the decision taken at week-16 was retrospectively defined as inappropriate if: (i) The decision was to switch to third-line, AND the resistance tests retrospectively showed that there was no resistance to the ongoing Protease Inhibitor (PI), AND the GSS was ≥ 2 ; OR (ii) The decision was to continue with second-line but the resistance tests retrospectively showed that there was resistance to the PI, OR the GSS was < 2 . When the baseline GSS was partly or entirely non-calculable (because of non-amplifiable sequences), the decision was a priori defined as appropriate, except for those who continued second-line, had amplifiable PR sequences showing that there was resistance to the ongoing PI, and had non amplifiable RT sequences making the GSS calculation impossible.

Statistical analysis

The percentage of patients with successful virological suppression at week-64 was calculated overall and for each group of decision taken at week-16. The numerator was the number of patients with a plasma HIV-1 RNA <50 copies per mL at week-64. The denominator was the total number of patients, overall and in each decision group. Patients who died, those who were lost to follow-up, and those who had a missing viral load value at week-64 were considered as failing. Patients who stayed on second-line at week-16 and then switched to third-line between week-16 and week-64 were analyzed in the group “stay on second-line”.

We looked for factors associated with (i) the decision taken at week-16 and (ii) successful virological suppression at week-64, using logistic regression univariate and then multivariate

models. Factors included in univariate analysis were chosen a priori (see the list in the Supplemental Appendix, page 3). All factors associated with the outcome with a $p < 0.25$ in univariate analysis were included in a full multivariate model. We used a stepwise descending procedure from the full model to reach the final model.

The medication possession ratio (MPR) was defined as the number of daily doses of antiretroviral drugs dispensed by the pharmacy to each patient between inclusion and week-64, divided by the patient's total follow-up time in days between inclusion and week-64.

Role of the funding source

The study sponsors, ANRS, had no role in study design; in the collection, analysis, and interpretation of data; in the writing of the report; nor in the decision to submit the paper for publication. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication. Data and codes will be made available from the corresponding author upon reasonable request.

Results

Between March 28, 2013 and May 11, 2015, 201 participants were included. Three (1.5%) were further excluded from the analysis because they did not meet all the inclusion criteria, and 198 (98.5%) were analyzed (Figure 2). Baseline characteristics are detailed in Table 1.

Participants were followed for a mean of 61 weeks (standard deviation [SD] 11 weeks). During follow-up, 2951/3024 (97.6%) scheduled monthly protocol visits were actually attended; 923/941 (98.1%) of scheduled quarterly viral load (VL) measurements were actually performed; the mean Medication Possession Ratio (MPR) was 94.8% (SD 9.1) overall, 96% (12.3) between inclusion and week 16, and 94.1% (13) between week-17 and week-64; the percentage of patients with a MPR $> 95\%$ was 53% overall, 64% between inclusion and week-16 and 54% between week-17 and week-64 ($p = 0.15$).

The mean number of adherence reinforcement interventions chosen decreased over time, from 4.9 (SD 1.7) per patient at baseline to 3.7 (SD 2.2) per patient at week-64 ($p < 0.001$) (Figure 3A). The most frequently chosen as well as stable interventions over time were pillboxes, weekly phone calls, alarm reminders, and DOT by a relative. Home visits, SMS reminders, support groups and individual sessions were less often selected and their choice tended to decrease over time (mixed effects logistic regression : $p < 0.0001$) (Figure 3B and 3C).

The decision taken at week-16 (based on week-12 VL) was to switch to third-line in 63 patients (33%) and to continue second-line in 130 (67%). Of the latter, 9 patients then switched to third-

line between week-16 and week-64 based on findings of repeated detectable VL after week-16. 64 (49%) of the 130 patients who continued second-line and 37 (59%) of the 63 patients who switched to third-line at week-16 had a VL<50 copies per mL at week-64 (Figure 4A). The evolution of the CD4 count in the two week-16 decision groups is shown in Figure 4B.

After study termination, GRT were performed on baseline frozen samples. Plasma from 181 patients had successful RT (n=172) and/or PR (n=175) amplification and sequencing (Table 2). At baseline, 138 patients had resistance to at least one drug, including 88 who had resistance to at least one PI. The latter included 44 patients with resistance to the PI included in their current treatment regimen. The baseline GSS was <2 in 57 patients, between 2 and 2.5 in 36, equal to 3 in 73 patients, and non-calculable in 27. Based on this GSS, the decision taken at week-16 was retrospectively deemed appropriate in 75% of patients, including 73% of patients who switched to third-line and 76% of those who continued second-line at week-16. Of the 31 patients who were judged to be inappropriately kept on second-line at week-16, 5 more switched to third-line before week-64 on findings of repeated detectable VL, 2 died while still on second-line, and 24 were alive and still on second-line at week-64. Of the latter, the week-64 VL was <50 copies per mL in 9, 50-399 copies per mL in 11, and ≥ 400 copies per mL in 4.

Of the 77 patients who had a VL ≥ 50 copies per mL at week-64, 52 had amplifiable RT sequences (n=43) and/or PR sequences (n=45) at week-64, of whom 40 had resistance to at least one drug, including 20 who had resistance to at least one PI. The latter included two patients with resistance to the PI included in their current treatment regimen. Only one had resistance to raltegravir, but this was a polymorphic mutation (E157Q) already detected on the baseline sample. Of the 26 patients who continued second-line at week-16 and had a VL ≥ 50 copies per mL with at least one resistance mutation at week-64, 12 had new drug resistance mutations, including 6 with a decrease in GSS. Of the 13 patients who switched to third-line at week-16 and had a VL ≥ 50 copies per mL with at least one resistance mutation at week-64, only one accumulated resistance mutations with no decrease in GSS. Resistance mutations detected at baseline and week-64 are detailed in the Supplemental Appendix, pages 6-7.

Of the 78 patients who had a VL ≥ 50 copies per mL at week-64, 74 had antiretroviral plasma concentrations measurements, including 52 who were still on second-line at week-64 and 22 who were on third-line (details in the Supplemental Appendix, page 8-9). Of these 74 patients: 55 (74%) had plasma concentrations found adequate for all drugs and 19 (26%) had inadequate concentrations for at least one drug (second-line 8 [15%], third-line 11 [50%]); 46 were on LPV/r (including 39 with adequate and 7 with inadequate LPV/r concentration), 22 were on

DRV (including 11 with adequate and 11 with inadequate DRV concentration) and 6 were on ATV/r (including 5 with adequate and 1 with inadequate ATV/r concentration); 66 had a GRT on the same serum sample (second-line 48, third-line 18), including 39 (59%) who had virus resistance to at least one drug (second-line 25 [52%], third-line 14 [78%]). Among these 66 patients, 27/48 (56%) of those with adequate concentrations for all drugs had virus resistance to at least one drug, and 12/18 (67%) of those with inadequate concentrations for at least one drug had virus resistance to at least one drug ($p=0.44$)

In multivariable analysis, baseline GSS ≥ 2 (adjusted Odds Ratio [aOR] 4.95, 95%CI 2.46-9.99, $p<0.0001$), and baseline viral load $\leq 5\log_{10}$ copies per mL (aOR 2.43, 95%CI, 1.20-4.93, $p=0.01$) were significantly associated with the decision to maintain second-line at week-16. A shorter duration of first-line ART (<3 years *vs.* ≥ 3 years, aOR 2.20, 95%CI 1.11-4.39, $p=0.0002$), a stable sexual partner (aOR 4.29, 95%CI 1.98-9.32, $p=0.0004$), and the decision to switch to third-line at week-16 (aOR 2.07, 95%CI 1.00-4.30, $p=0.02$) were significantly associated with the probability of having a VL <50 copies per mL at week-64. In addition, there was an interaction between sex and resistance to NNRTI at baseline in the association with virological success at week-64. Women who had resistance to NNRTI at baseline were less likely to have a VL <50 copies per mL at week-64 than other women (aOR 0.18, 0.07-0.45, $p=0.0002$), whereas among patients who had no resistance to NNRTI at baseline, women were more likely than men to have a VL <50 copies per mL at week-64 (aOR 7.31, 95%CI 2.44-21.90, $p=0.0004$)(see details in the Supplemental Appendix, page 11-12).

During follow-up, there were 103 serious adverse events in 70 patients (see diagnoses in the Supplemental Appendix, page 13). 48 events occurred before week-16 and 55 after week-16, including 23 in patients who switched to third-line, and 32 in patients who continued second-line at week-16. One grade 2 and one grade 3 events were possibly related to a drug. None of these two events led to discontinuance of the drug. Ten out of 15 deaths were of unknown cause. The 5 identified causes of death were tuberculosis ($n=2$), severe sepsis, Kaposi's sarcoma, and unexplained central neurological syndrome.

Discussion

The studies conducted in sub-Saharan Africa reported rates of failure as high as 21% to 40% for second-line ART at 12 months (10). The main reasons for ART failure are poor adherence and drug resistance mutations.

Our findings confirm not only that poor adherence is the first problem to tackle when resistance tests are not routinely available, but also that it is effectively a manageable problem. In our study enhanced adherence was effective in leading to re-suppression in 67% of participants. This is consistent with studies performed in South Africa, in which re-suppression also occurred in more than 50% of patients failing second-line ART after adherence was reinforced (9,13). In our study, 29% of participants had no resistance mutations at baseline, only 20% had resistance to their ongoing PI, and 66% had a GSS ≥ 2 , all of which is consistent with the literature showing that poor adherence to PI-based therapy does not consistently lead to resistance patterns that require the use of third-line drugs (14).

Limited access to care centers, transportation issues, education level, repeated drug stock-out and the negative influence of traditional medicine were reported as major barriers to adherence optimization (15). Risk factors for poor adherence may vary by region (10,16,17). Our study in West Africa confirms the interest of combining counseling with adherence support interventions, and suggests that a comprehensive, patient-centered approach involving healthcare workers and the community is a strategy to encourage for patients with multiple treatment failure (18). In our study, the preferred adherence reinforcement measures were pill organizers, phone calls and alarm reminders. These measures were previously associated with improved adherence (19). Previous reports also suggested that it could be important to promote ART regimens that are better tolerated and easier to use with fewer tablets, in order to prevent failure rather than having to treat it (20).

Our results provide the first data on the use of darunavir plus raltegravir-based third-line therapy in West Africa. They are consistent with data from Southern Africa and industrialized countries that reports virological success rates of 58% to 80% at 6 to 12 months, using a threshold of 50 copies per mL (12,21–23). The highest rates of virological suppression were obtained when treatment adaptation was guided by genotypic resistance tests (12,24). In our study resistance tests were performed after the end of the study, and the results suggest that the decision to switch blindly to third-line or remain on second-line was appropriate in 75% of patients. We have previously reported that this strategy could be cost-effective (25).

Third-line ART tolerance was excellent, with no serious adverse events requiring treatment discontinuation, as previously reported in other studies using darunavir and raltegravir-containing third-line therapies (12,21,25). We did not observe neuropsychiatric events such as sleep disturbance, agitation, and headaches (26–28). At the initiation of the study, raltegravir was the only available INSTI option.

Previous studies reported to 5% to 12% of patients with prolonged failure of a LPV/r or ATV/r-based ART have a DRV cross-resistance (29–31). In our study, 12% of participants had resistance to DRV QD but only 0.5% had resistance to DRV BID at baseline. This suggests that our strategy could be interesting in settings where routine genotypic resistance testing is not available and where LPV/r or ATV/r is the most frequent boosted PI used in second-line treatment, provided DRV BID is preferred over DRV QD (4,5).

Our study have the following limitations. First, our patients visited the clinic monthly, with viral load testing every 3 months. This was feasible in the context of a capital city. In remote areas, the tendency is rather to reduce the number of clinic visits which are both time and money-consuming. Therefore, our results are not generalizable to all settings. Second, this is a non-comparative study, which aim was to explore the challenges of deciding to switch to third-line ART in settings with limited access to GRT. It was not meant to assess whether making a decision without the help of GRT was as effective as using GRT. Even if GRT still has a prohibitive cost as well as a long turnaround time partly due to low demand (5,29), there is no doubt that the use of GRT is the gold standard for informing the choice of optimized third-line ART for patients with multidrug-resistant viruses (29,30).

In conclusion, in settings where genotypic resistance tests are not routinely available, a standardized algorithm based on intensive adherence reinforcement and repeated viral load monitoring appears to be a reasonable strategy for managing patients on second-line ART with virological failure. In sub-Saharan Africa, lack of access to resistance testing should no longer be an obstacle to the prescription of third-line ART.

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All other authors declared no conflict of interest.

Authors contribution

XA, SPE, PMG and RL designed the research. AA, MC, ID, JD, MD, SPE, FE, DM, EM, RM, AS, MS, and JZ recruited and followed the participants. AB, DG, SK, JLC, LAM, and RM coordinated the data management and study monitoring. GB, MLC, MCO, AIM, LS, CTK and TAT undertook the virological tests. GP undertook the pharmacological tests. XA, DG, RM analysed the data. All authors reviewed the final report. XA, MLC, SE, PMG and RL drafted the manuscript. All authors critically revised the manuscript and have read and approved the final manuscript.

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References

1. WHO/UNAIDS/UNICEF. Towards Universal Access, Progress Report 2018. Available from: <http://www.who.int/hiv/pub/progressreports/en/>
2. Estill J, Ford N, Salazar-Vizcaya L, Haas AD, Blaser N, Habiyambere V, et al. The need for second-line antiretroviral therapy in adults in sub-Saharan Africa up to 2030: a mathematical modelling study. *Lancet HIV* 2016; 3(3):e132-139.
3. Haas AD, Keiser O, Balestre E, Brown S, Bissagnene E, Chimbetete C, et al. Monitoring and switching of first-line antiretroviral therapy in adult treatment cohorts in sub-Saharan Africa: collaborative analysis. *Lancet HIV* 2015; 2(7):e271-278.
4. Jordan MR, Bennett DE, Wainberg MA, Havlir D, Hammer S, Yang C, et al. Update on World Health Organization HIV drug resistance prevention and assessment strategy: 2004-2011. *Clin Infect Dis* 2012; 54 Suppl 4:S245-249.
5. Lessells RJ, Avalos A, de Oliveira T. Implementing HIV-1 genotypic resistance testing in antiretroviral therapy programs in Africa: needs, opportunities, and challenges. *AIDS Rev* 2013; 15(4):221-9.
6. Sethi AK, Celentano DD, Gange SJ, Moore RD, Gallant JE. Association between adherence to antiretroviral therapy and human immunodeficiency virus drug resistance. *Clin Infect Dis* 2003; 37(8):1112-8.
7. Onoya D, Nattey C, Budgell E, van den Berg L, Maskew M, Evans D, et al. Predicting the Need for Third-Line Antiretroviral Therapy by Identifying Patients at High Risk for Failing Second-Line Antiretroviral Therapy in South Africa. *AIDS Patient Care STDS* 2017; 31(5):205-12.
8. Sigaloff KCE, Hamers RL, Wallis CL, Kityo C, Siwale M, Ive P, et al. Second-line antiretroviral treatment successfully resuppresses drug-resistant HIV-1 after first-line failure: prospective cohort in Sub-Saharan Africa. *J Infect Dis* 2012; 205(11):1739-44.
9. Garone DB, Conradie K, Patten G, Cornell M, Goemaere W, Kunene J, et al. High rate of virological re-suppression among patients failing second-line antiretroviral therapy following enhanced adherence support: A model of care in Khayelitsha, South Africa. *Southern African Journal of HIV Medicine* 2013; 14(4):170-6.

- 10 Mills EJ, Lester R, Thorlund K, Lorenzi M, Muldoon K, Kanter S, et al. Interventions to promote adherence to antiretroviral therapy in Africa: a network meta-analysis. *Lancet HIV* 2014;1(3):e104-111.
- 11 Jung BH, Rezk NL, Bridges AS, Corbett AH, Kashuba ADM. Simultaneous determination of 17 antiretroviral drugs in human plasma for quantitative analysis with liquid chromatography–tandem mass spectrometry. *Biomedical Chromatography* 2007; 21(10):1095–104.
- 12 Onoya D, Brennan AT, Berhanu R, van der Berg L, Buthelezi T, Fox MP. Changes in second-line regimen durability and continuity of care in relation to national ART guideline changes in South Africa. *J Int AIDS Soc* 2016; 19(1):20675.
- 13 Sigaloff KCE, Hamers RL, Wallis CL, Kityo C, Siwale M, Ive P, et al. Second-line antiretroviral treatment successfully resuppresses drug-resistant HIV-1 after first-line failure: prospective cohort in Sub-Saharan Africa. *J Infect Dis* 2012; 205(11):1739–44.
- 14 Collier D, Iwuji C, Derache A, de Oliveira T, Okesola N, Calmy A, et al. Virological Outcomes of Second-line Protease Inhibitor-Based Treatment for Human Immunodeficiency Virus Type 1 in a High-Prevalence Rural South African Setting: A Competing-Risks Prospective Cohort Analysis. *Clin Infect Dis* 2017; 64(8):1006–16.
- 15 Heestermaans T, Browne JL, Aitken SC, Vervoort SC, Klipstein-Grobusch K. Determinants of adherence to antiretroviral therapy among HIV-positive adults in sub-Saharan Africa: a systematic review. *BMJ Glob Health* 2016; 1(4):e000125.
- 16 Okoror TA, Falade CO, Olorunlana A, Walker EM, Okareh OT. Exploring the cultural context of HIV stigma on antiretroviral therapy adherence among people living with HIV/AIDS in southwest Nigeria. *AIDS Patient Care STDS* 2013; 27(1):55–64.
- 17 Bijker R, Jiamsakul A, Kityo C, Kiertiburanakul S, Siwale M, Phanuphak P, et al. Adherence to antiretroviral therapy for HIV in sub-Saharan Africa and Asia: a comparative analysis of two regional cohorts. *J Int AIDS Soc*. 2017 Mar 3;20(1):21218.
- 18 Kelly JD, Frankfurter R, Lurton G, Conteh S, Empson SF, Daboh F, et al. Evaluation of a community-based ART programme after tapering home visits in rural Sierra Leone: a 24-month retrospective study. *SAHARA J* 2018; 15(1):138–45.

- 19 KanTERS S, Park JJH, Chan K, Socias ME, Ford N, Forrest JI, et al. Interventions to improve adherence to antiretroviral therapy: a systematic review and network meta-analysis. *Lancet HIV* 2017; 4(1):e31–40.
- 20 Paton NI, Kityo C, Thompson J, Nankya I, Bagenda L, Hoppe A, et al. Nucleoside reverse-transcriptase inhibitor cross-resistance and outcomes from second-line antiretroviral therapy in the public health approach: an observational analysis within the randomised, open-label, EARNEST trial. *Lancet HIV* 2017; 4(8):e341–8.
- 21 Meintjes G, Dunn L, Coetsee M, Hislop M, Leisegang R, Regensberg L, et al. Third-line antiretroviral therapy in Africa: effectiveness in a Southern African retrospective cohort study. *AIDS Res Ther* 2015; 12:39.
- 22 Moorhouse M, Maartens G, Francois Venter WD, Moosa M-Y, Steegen K, Jamaloodien K, et al. Third-line antiretroviral therapy programme in the South African public sector: cohort description and virological outcomes. *J Acquir Immune Defic Syndr* 2019; 80(1):73–78.
- 23 Chimbetete C, Katzenstein D, Shamu T, Spoerri A, Estill J, Egger M, et al. HIV-1 Drug Resistance and Third-Line Therapy Outcomes in Patients Failing Second-Line Therapy in Zimbabwe. *Open Forum Infect Dis* 2018; 5(2): 1-5.
- 24 Ouattara EN, Ross EL, Yazdanpanah Y, Wong AY, Robine M, Losina E, et al. Clinical impact and cost-effectiveness of making third-line antiretroviral therapy available in sub-Saharan Africa: a model-based analysis in Côte d’Ivoire. *J Acquir Immune Defic Syndr* 2014; 66(3):294–302.
- 25 Arastéh K, Yeni P, Pozniak A, Grinsztejn B, Jayaweera D, Roberts A, et al. Efficacy and safety of darunavir/ritonavir in treatment-experienced HIV type-1 patients in the POWER 1, 2 and 3 trials at week- 96. *Antivir Ther (Lond)* 2009; 14(6):859–64.
- 26 Elzi L, Erb S, Furrer H, Cavassini M, Calmy A, Vernazza P, et al. Adverse events of raltegravir and dolutegravir. *AIDS* 2017; 31(13):1853–8.
- 27 KanTERS S, Socias ME, Paton NI, Vitoria M, Doherty M, Ayers D, et al. Comparative efficacy and safety of second-line antiretroviral therapy for treatment of HIV/AIDS: a systematic review and network meta-analysis. *Lancet HIV* 2017; 4(10):e433–41.
- 28 Lepik KJ, Yip B, Ulloa AC, Wang L, Toy J, Akagi L, et al. Adverse drug reactions to integrase strand transfer inhibitors. *AIDS* 2018; 32(7):903–12.

- 29 Inzaule SC, Ondoa P, Peter T, Mugenyi PN, Stevens WS, Wit TFR de, et al. Affordable HIV drug-resistance testing for monitoring of antiretroviral therapy in sub-Saharan Africa. *The Lancet Infectious Diseases* 2016; 16(11):e267–75.
- 30 Maiga AI, Fofana DB, Cisse M, Diallo F, Maiga MY, Traore HA, et al. Characterization of HIV-1 antiretroviral drug resistance after second-line treatment failure in Mali, a limited-resources setting. *J Antimicrob Chemother* 2012; 67(12):2943–8.
- 31 Van Zyl GU, Liu TF, Claassen M, Engelbrecht S, de Oliveira T, Preiser W, et al. Trends in Genotypic HIV-1 Antiretroviral Resistance between 2006 and 2012 in South African Patients Receiving First- and Second-Line Antiretroviral Treatment Regimens. *PLoS ONE* 2013;8(6):e67188.

Table 1: Baseline characteristics (n=198)

Age, years, median (IQR)	41	(36-48)
Women, n (%)	136	(69)
Men, n (%)	62	(31)
History of adherence reinforcement in the last 6 months n (%)	109	(55)
WHO clinical stage 3 or 4, n (%)	157	(79)
Positive plasma HBs Ag, n (%)	16	(8)
Baseline CD4 count per μL, median (IQR)	238	(134-372)
CD4 nadir, count per μL, median (IQR)	149	(66-277)
Plasma HIV-1 RNA, log₁₀ copies/ml, median (IQR)	4.5	(3.6-5.1)
Ongoing ART regimen, n (%)		
2 NRTIs[†] + lopinavir/ritonavir	171	(86)
2 NRTIs[†] + atazanavir/ritonavir	27	(14)
Previous time on ART, years, median (IQR)		
Time on 1st line	3	(2-5)
Time on 2nd line	3	(2-6)
Adherence Reinforcement measures chosen by the patient		
Pill organizer	185	(93%)
Weekly phone call	170	(86%)
Alarm reminders	146	(74%)
DOT by a relative	123	(62%)
SMS reminders	96	(48%)
Home visits	112	(57%)
Support group	77	(39%)
Individual sessions	53	(27%)
Non-ART drug limitation	13	(7%)

Legend of Table 1:

n= number; IQR: interquartile range; ART: Antiretroviral Therapy; HBs Ag: hepatitis B surface antigen; DOT: directly observed treatment; SMS: Short Message System; NRTIs: Nucleoside Reverse Transcriptase Inhibitors; [†] The 2 NRTIs were tenofovir-XTC in 103 patients (52%), abacavir-lamivudine in 50 patients (25%) and zidovudine- lamivudine in 45 patients (23%)

Table 2: Drug resistance at baseline and end of follow-up (N=193)

	BASELINE (Day-0) †						END OF FOLLOW-UP (Week-64) ‡					
	Overall		According to week-16 decision				Overall		According to week-16 decision			
			Switch to 3 rd -line		Maintain 2 nd -line				Switch to 3 rd -line		Maintain 2 nd -line	
<u>Viral load >50 copies/ml</u>	193	(100%)	63	(100%)	130	(100%)	77	(100%)	21	(100%)	56	(100%)
≥50 <400 copies/ml	0	-	0	-	0	-	46	(60%)	7	(33%)	39	(70%)
≥400 copies/ml	193	(100%)	63	(100%)	130	(100%)	31	(40%)	14	(67%)	17	(30%)
<u>Genotype Resistance Tests</u>												
Available for at list one group	181	(100%)	61	(100%)	120	(100%)	52	(100%)	17	(100%)	35	(100%)
At least one resistance	138	(76%)	54	(89%)	84	(70%)	40	(77%)	14	(82%)	26	(74%)
Available for NRTIs	172	(100%)	59	(100%)	113	(100%)	43	(100%)	15	(100%)	28	(100%)
At least one resistance	95	(55%)	39	(66%)	56	(50%)	25	(58%)	4	(27%)	21	(75%)
Resistance to:												
3TC/FTC	88	(51%)	37	(63%)	51	(45%)	23	(53%)	4	(27%)	19	(68%)
ZDV	43	(25%)	22	(37%)	21	(19%)	13	(30%)	4	(27%)	9	(32%)
d4T	50	(29%)	26	(44%)	24	(21%)	15	(35%)	4	(27%)	11	(39%)
ddI	23	(13%)	10	(17%)	13	(12%)	4	(9%)	0	(0%)	4	(14%)
ABC	46	(27%)	24	(41%)	22	(19%)	14	(32%)	3	(20%)	11	(39%)
TDF	23	(13%)	17	(29%)	6	(5%)	6	(14%)	2	(13%)	4	(14%)

Table 2: Drug resistance at baseline and end of follow-up (N=193) (continued)

	BASELINE (Day-0) †						END OF FOLLOW-UP (Week 64) ‡					
	Overall		According to week-16 decision				Overall		According to week-16 decision			
			Switch to 3 rd -line		Maintain 2 nd -line				Switch to 3 rd -line		Maintain 2 nd -line	
Available for NNRTIs	172	(100%)	59	(100%)	113	(100%)	43	(100%)	15	(100%)	28	(100%)
At least one resistance	92	(53%)	36	(61%)	56	(50%)	27	(63%)	9	(60%)	18	(64%)
Resistance to:												
EFV and/or NVP	87	(51%)	32	(54%)	55	(49%)	24	(56%)	7	(47%)	17	(61%)
RPV	50	(29%)	22	(37%)	28	(25%)	16	(37%)	4	(27%)	12	(43%)
ETR	34	(20%)	16	(27%)	18	(16%)	10	(23%)	2	(13%)	8	(29%)
Available for PIs	175	(100%)	60	(100%)	115	(100%)	45	(100%)	15	(100%)	30	(100%)
At least one resistance	88	(50%)	46	(77%)	42	(37%)	20	(44 %)	7	(47%)	13	(43%)
Resistance to:												
LPV	39	(22%)	29	(48%)	9	(8%)	3	(7%)	1	(1%)	2	(7%)
ATV/r	21	(12%)	16	(27%)	5	(4%)	4	(9%)	2	(1%)	2	(7%)
DRV/r QD	23	(13%)	18	(30%)	5	(4%)	1	(2%)	0	(0%)	1	(3%)
DRV/r BID	1	(0.6%)	1	(2%)	0	(0%)	0	-	0	(0%)	0	(0%)
<u>Genotypic Sensitivity Score ¶¶</u>	193	(100%)	63	(100%)	130	(100%)	77	(100%)	21	(100%)	56	(100%)
Non calculable	27	(14%)	5	(8%)	22	(17%)	42	(55%)	9	(43%)	33	(59%)
Calculable	166	(86%)	58	(92%)	108	(83%)	35	(45%)	12	(57%)	23	(41%)
< 2.0	57	(34%)	32	(55%)	25	(23%)	8	(23%)	0	(0%)	8	(35%)
2 to 2.5	36	(22%)	8	(14%)	28	(26%)	13	(37%)	2	(17%)	11	(48%)
3.0	73	(44%)	18	(31%)	55	(51%)	6	(17%)	3	(25%)	3	(13%)
4.0	-	-	-	-	-	-	8	(23%)	7	(58%)	1	(4%)
<u>Week-16 decision</u>	193	(100%)	63	(100%)	130	(100%)	-	-	-	-	-	-
Appropriate	145	(75%)	46	(73%)	99	(76%)	-	-	-	-	-	-
Inappropriate	48	(25%)	17	(27%)	31	(24%)	-	-	-	-	-	-

Legend of table 2

† Baseline corresponds to the tests performed after the end of the study on plasma samples that had been taken and frozen on inclusion.

‡ End of follow-up corresponds to the tests performed after the end of the study on plasma samples taken at week 44 in patients who had a viral load >50 copies/ml at Week 64. Of the 198 study participants, 5 died before week 16 and 193 were alive at week 16. Only the latter are detailed in this table.

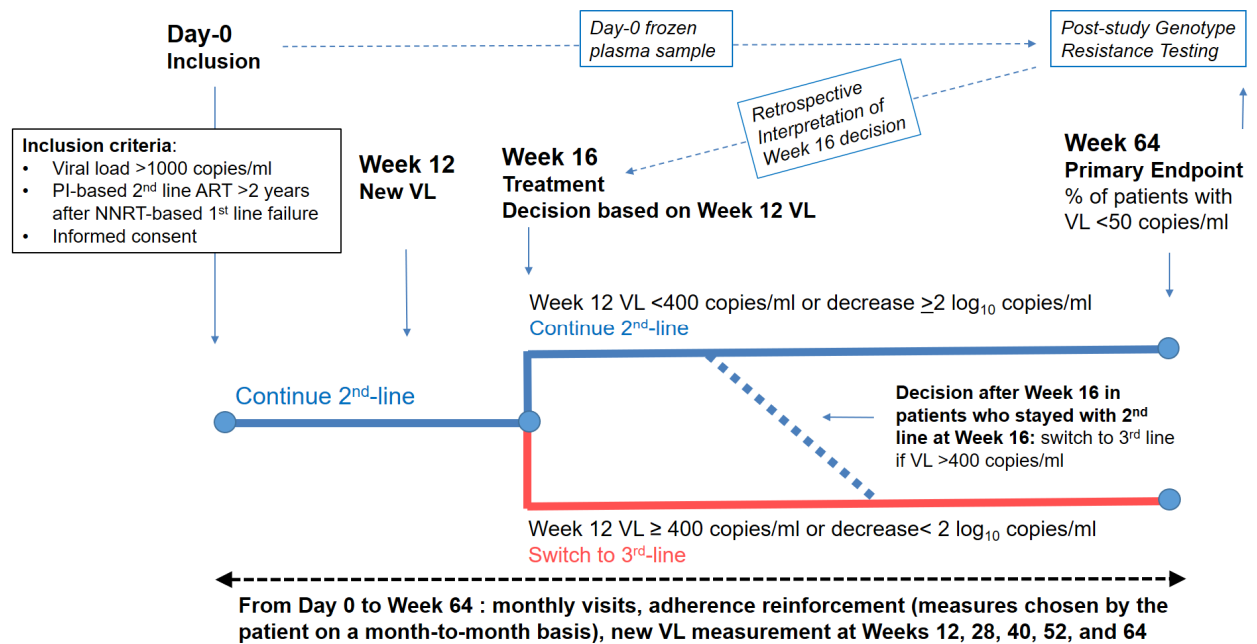
¶¶ No patient had a GSS=3.5

NRTIs: Nucleoside Reverse Transcriptase Inhibitors; NNRTIs: Non-Nucleoside Reverse Transcriptase Inhibitors; PIs: Protease Inhibitors; ABC: abacavir, TDF: tenofovir, d4T: stavudine; ddI: didanosine; LPV: lopinavir, ATV/r: atazanavir/ritonavir, DRV/r QD: darunavir/ritonavir single dose, DRV/r BID: darunavir/ritonavir twice a day, EFV: efavirenz, NVP: nevirapine, RPV: rilpivirine, ETR: etravirine.

Resistance to Integrase Strand Transfer Inhibitors (INSTIs) were tested at week 64 in the 17 patients who received INSTIs and had VL \geq 400 copies/ml. Only one patient had a resistance mutation (E157Q). This mutation was already detectable at baseline

.

Figure 1: Study design

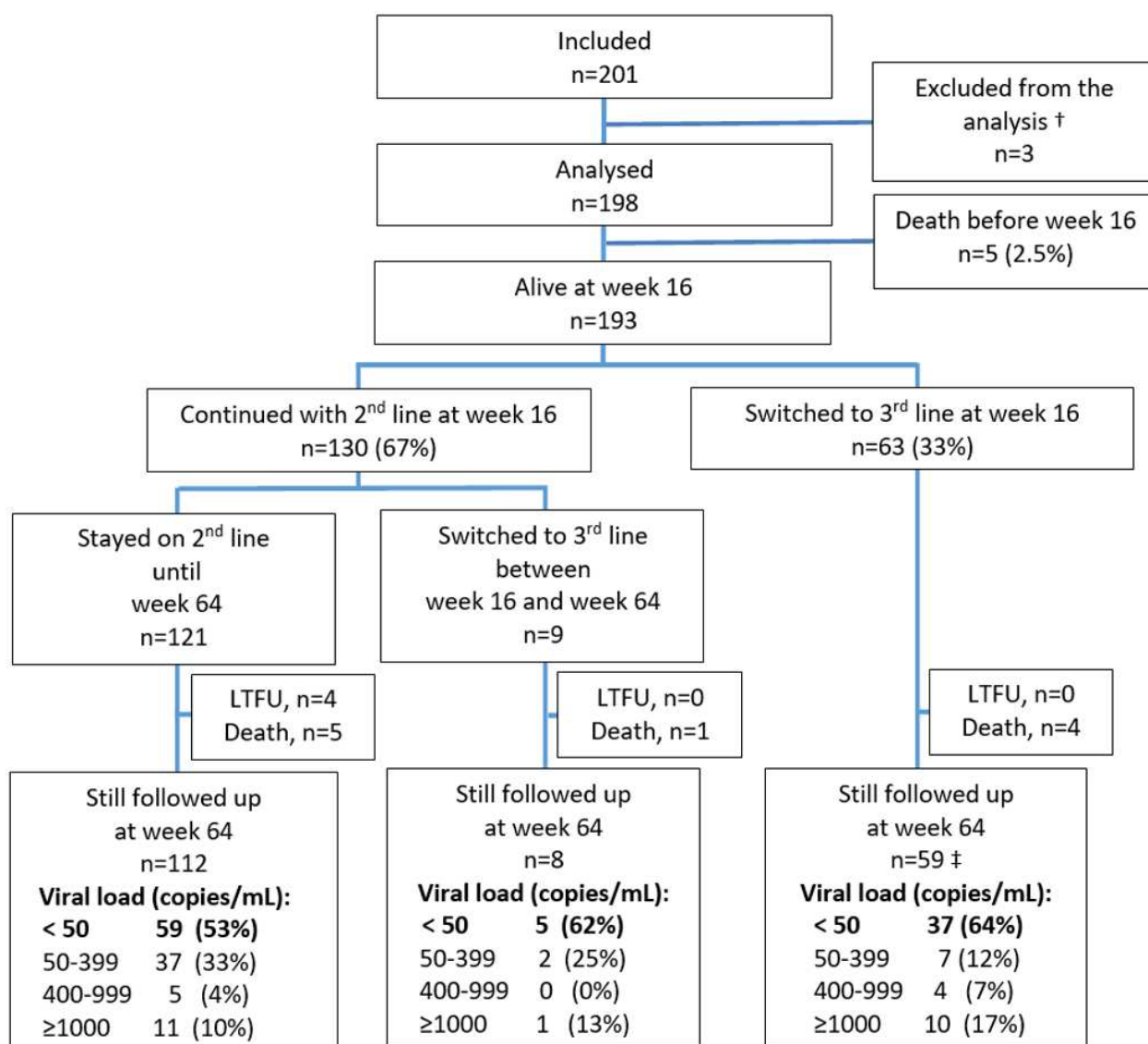


Legend of Figure 1

NNRTI: Non Nucleoside Reverse Transcriptase Inhibitor

VL: Plasma HIV-1 RNA (viral load)

Figure 2: Flow Chart



Legend of Figure 2

NNRTI: Non Nucleoside Reverse Transcriptase Inhibitor

LTFU: Lost-to-follow-up

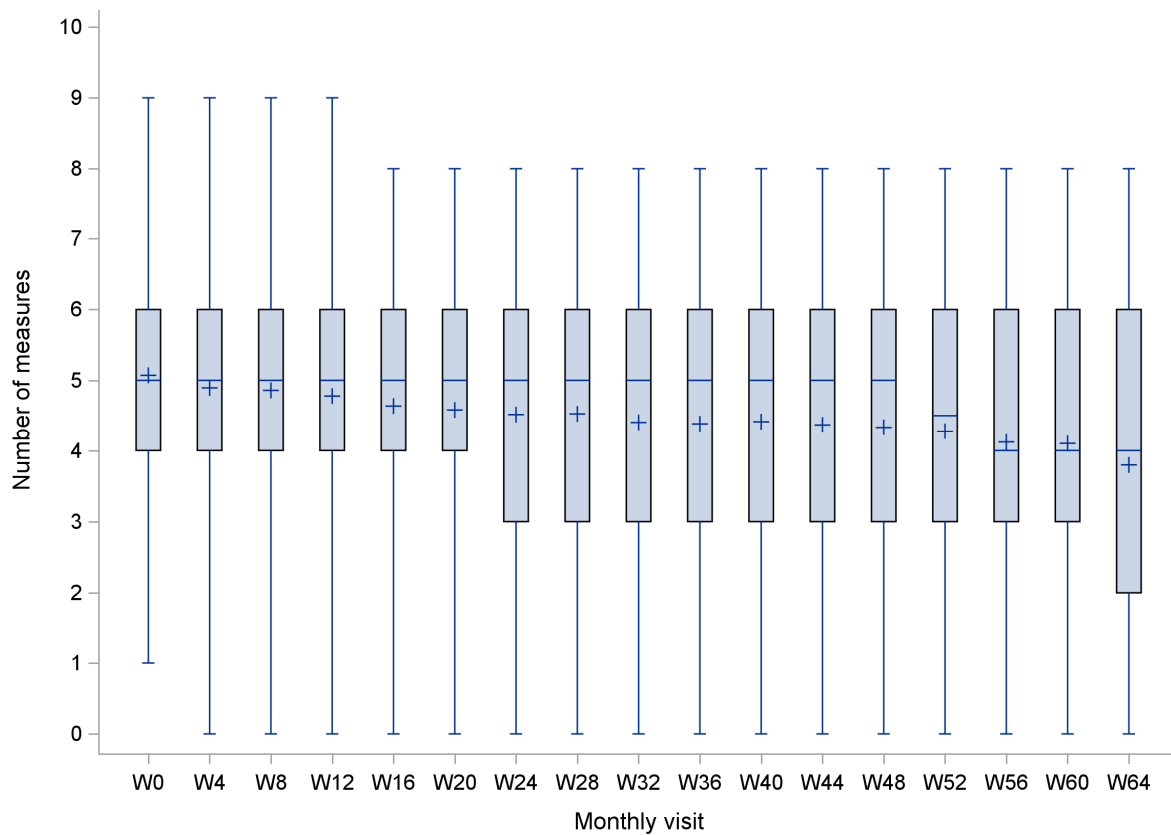
VL: Plasma HIV-1 RNA (viral load)

† Three patients were excluded from the analysis because their first-line ART was a PI-based regimen.

‡ One missing VL value

Figure 3: Adherence reinforcement measures chosen by the participants over time

Figure 3A: Number of measures per participant



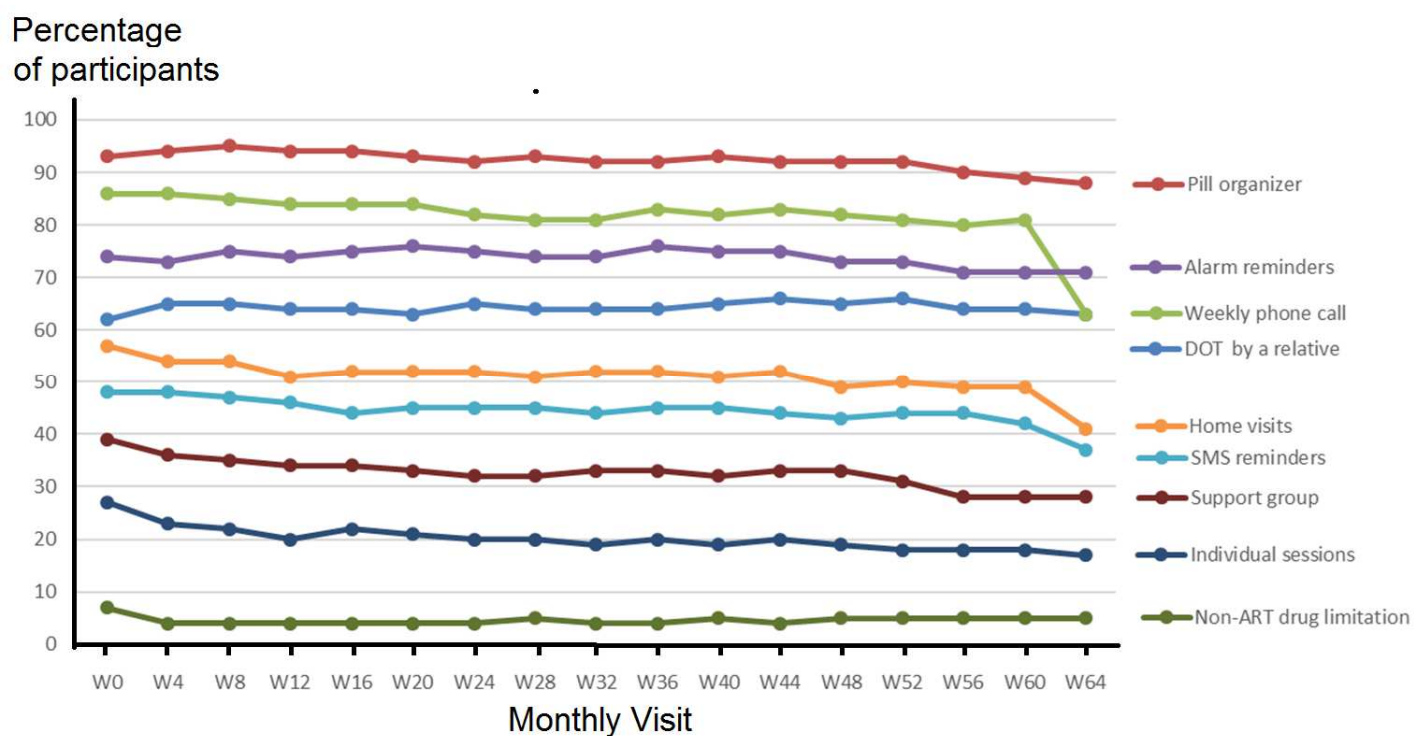
Legend of Figure 3A

The figure displays the distribution of the number of adherence reinforcement measures chosen per patient at each monthly visit.

The central rectangle spans the first quartile to the third quartile (the interquartile range). The segment inside the rectangle shows the median. The extremities of the bars above and below the box show the locations of the minimum and maximum. The cross shows the mean value.

W : Week.

Figure 3B: Month-to-month choice



Legend of Figure 3B

The figure displays the percentage of participants who chose each intervention on a given monthly visit from the total number of participants

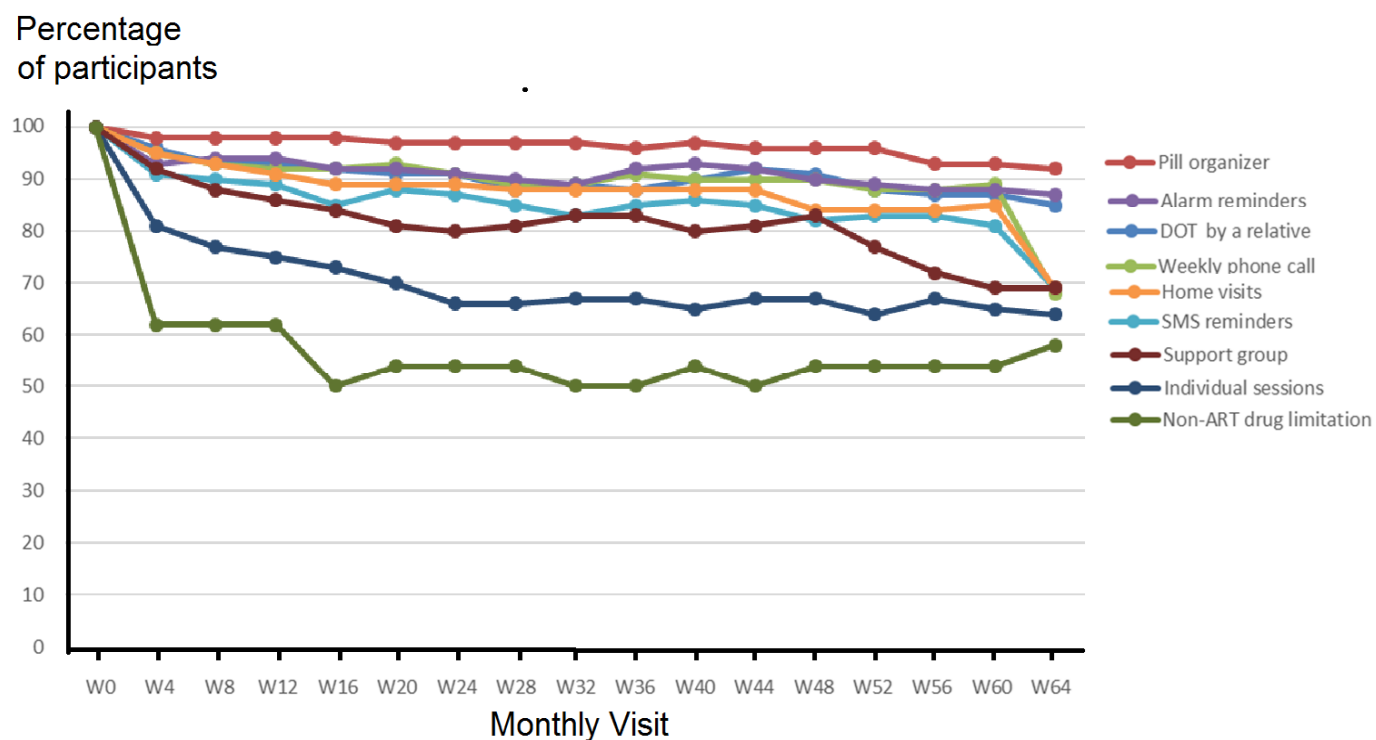
DOT: Directly Observed Treatment

SMS: Short Message System

W: Week

ART: Antiretroviral therapy

Figure 3C: Stick to the initial choice



Legend of Figure 3C

The figure displays the percentage of participants who chose a given intervention on a given monthly visit from the number of participants who selected the intervention at baseline.

DOT: Directly Observed Treatment

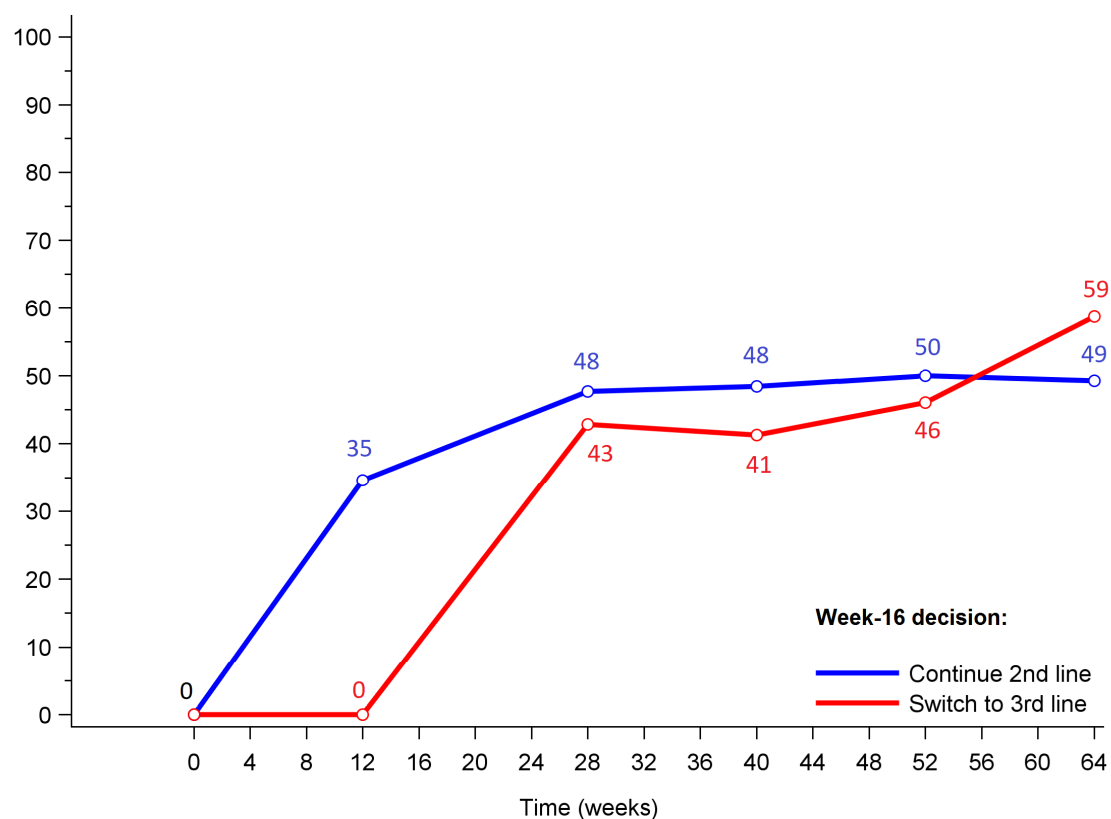
SMS: Short Message System

W: Week

ART: Antiretroviral therapy

Figure 4A: Virologic resuppression over time, according to week-16 decision

Percentage of patients with
plasma HIV-1 RNA <50 copies/ml



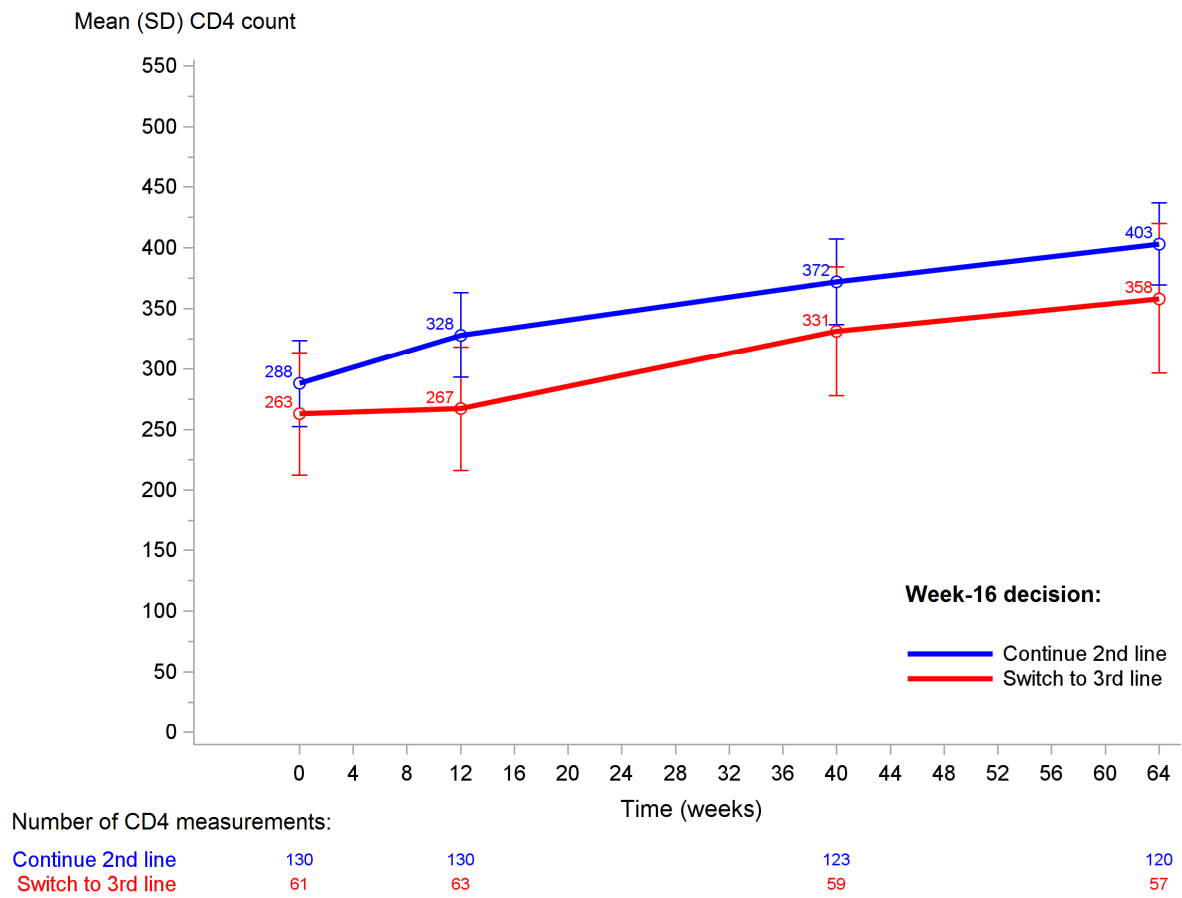
VL (copies/ml)	W0	W12		W28		W40		W52		W64	
	N	N	%	N	%	N	%	N	%	N	%
Continue 2nd line											
<50	0	45	35%	62	48%	63	48%	65	50%	64	49%
50-1000	0	73	56%	44	34%	40	31%	46	35%	44	34%
>1000	130	12	9%	21	16%	20	15%	10	8%	12	9%
Missing †	0	0	0%	3	2%	7	5%	9	7%	10	8%
Switch to 3rd line											
<50	0	0	0%	27	43%	26	41%	29	46%	37	59%
50-1000	0	6	10%	22	35%	22	35%	20	32%	11	17%
>1000	63	57	90%	12	19%	11	17%	10	16%	10	16%
Missing †	0	0	0%	2	3%	4	6%	4	6%	5	8%

Legend of Figure 4A

W: week; N: number; %: percentage

† Missing includes participants dead, lost to follow-up, or still in active follow-up but with missing VL value.

Figure 4B: CD4 count distribution over time, according to week-16 decision



Legend of Figure 4B

SD: standard deviation