Annals of Internal Medicine ORIGINAL RESEARCH

Resistance Testing for Management of HIV Virologic Failure in Sub-Saharan Africa

An Unblinded Randomized Controlled Trial

Mark J. Siedner, MD; Mahomed-Yunus S. Moosa, MBChB; Suzanne McCluskey, MD; Rebecca F. Gilbert, BA; Selvan Pillay, MSc; Isaac Aturinda, MBA; Kevin Ard, MD; Winnie Muyindike, MMed; Nicholas Musinguzi, MS; Godfrey Masette, BSc; Melendhran Pillay, MSc; Pravikrishnen Moodley, PhD; Jaysingh Brijkumar, MBBS; Tamlyn Rautenberg, PhD; Gavin George, PhD; Rajesh T. Gandhi, MD; Brent A. Johnson, PhD; Henry Sunpath, MBChB*; Mwebesa B. Bwana, MBChB*†; and Vincent C. Marconi, MD*

Background: Virologic failure in HIV predicts the development of drug resistance and mortality. Genotypic resistance testing (GRT), which is the standard of care after virologic failure in high-income settings, is rarely implemented in sub-Saharan Africa.

Objective: To estimate the effectiveness of GRT for improving virologic suppression rates among people with HIV in sub-Saharan Africa for whom first-line therapy fails.

Design: Pragmatic, unblinded, randomized controlled trial. (ClinicalTrials.gov: NCT02787499)

Setting: Ambulatory HIV clinics in the public sector in Uganda and South Africa.

Patients: Adults receiving first-line antiretroviral therapy with a recent HIV RNA viral load of 1000 copies/mL or higher.

Intervention: Participants were randomly assigned to receive standard of care (SOC), including adherence counseling sessions and repeated viral load testing, or immediate GRT.

Measurements: The primary outcome of interest was achievement of an HIV RNA viral load below 200 copies/mL 9 months after enrollment.

Results: The trial enrolled 840 persons, divided equally between countries. Approximately half (51%) were women. Most (72%) were receiving a regimen of tenofovir, emtricitabine, and efavirenz at enrollment. The rate of virologic suppression did not differ 9 months after enrollment between the GRT group (63% [263 of 417]) and SOC group (61% [256 of 423]; odds ratio [OR], 1.11 [95% CI, 0.83 to 1.49]; P = 0.46). Among participants with persistent failure (HIV RNA viral load ≥1000 copies/mL) at 9 months, the prevalence of drug resistance was higher in the SOC group (76% [78 of 103] vs. 59% [48 of 82]; OR, 2.30 [CI, 1.22 to 4.35]; P = 0.014). Other secondary outcomes, including 9-month survival and retention in care, were similar between groups.

Limitation: Participants were receiving nonnucleoside reverse transcriptase inhibitor–based therapy at enrollment, limiting the generalizability of the findings.

Conclusion: The addition of GRT to routine care after first-line virologic failure in Uganda and South Africa did not improve rates of resuppression.

Primary Funding Source: The President's Emergency Plan for AIDS Relief and the National Institute of Allergy and Infectious Diseases.

Ann Intern Med. 2021;174:1683-1692. doi:10.7326/M21-2229 **Annals.org**
For author, article, and disclosure information, see end of text.
This article was published at Annals.org on 26 October 2021.

* Drs. Sunpath, Bwana, and Marconi contributed equally to this work.
† Deceased.

Virologic failure in HIV remains a major public health threat in sub-Saharan Africa. It occurs in approximately 10% to 30% of patients within 2 years of antiretroviral therapy (ART) initiation and is associated with development of drug resistance, increased risk for opportunistic infections and death, and ongoing transmission of HIV (1–6).

The optimal management of virologic failure is unknown. Guidelines published by the U.S. Department of Health and Human Services recommend genotypic resistance testing (GRT) to assist in the management of virologic failure (7). Small randomized trials and observational studies completed in the early ART era in the United States suggested that GRT has benefit in virologic control and selection of active regimens (8–11). However, GRT after failure of first-line therapy is not routinely supported by treatment guidelines by the World Health Organization and is not typically done in most sub-Saharan African countries (12).

However, increasing relative costs of second-line therapy compared with first-line therapy, declining costs of GRT, and increasing availability of GRT in the region have changed this landscape (13, 14). Moreover, an immediate genotypic resistance strategy might expedite management of treatment failure, which currently includes a prolonged process of adherence support, repeated viral load testing, and result reporting and is associated with high rates of loss to follow-up (3, 15–17). Finally, GRT might also serve as an adherence support intervention by providing

See also:

Editorial comment. 1753

Web-Only
Supplement

patients and providers with objective data on whether virologic failure is due to resistance or adherence challenges, and thus helping to identify the most appropriate response. Nevertheless, whether GRT will improve outcomes or reduce costs compared with current practice is unknown.

We did an unblinded, randomized controlled trial of persons receiving first-line ART with virologic failure in public-sector clinics in Uganda and South Africa. The trial aimed to estimate the effectiveness of GRT for improving management of virologic failure in the sub-Saharan African region. We hypothesized that the addition of GRT to routine care would improve rates of virologic suppression 9 months later.

METHODS

Study Design

The REVAMP (Resistance Testing Versus Adherence Support for Management of Patients with Virologic Failure on First-Line Antiretroviral Therapy in sub-Saharan Africa) trial was a pragmatic, unblinded, parallel-group, randomized controlled trial (ClinicalTrials.gov: NCT02787499). It was designed to estimate the effectiveness and cost-effectiveness of adding GRT to routine care in the public sector in sub-Saharan Africa. The complete trial protocol is available in the **Supplement** (available at Annals.org) (18). The trial was approved by the institutional review committees at the Mbarara University of Science and Technology, the Uganda National Council for Science and Technology, the University of KwaZulu-Natal, and Mass General Brigham. All participants provided signed informed consent.

Eligibility Criteria, Recruitment, and Randomization

Potential participants were recruited from 1 of 5 public-sector HIV clinics in Mbarara, Uganda (Mbarara Regional Referral Hospital Immune Suppression Syndrome Clinic), or Durban, South Africa (Addington Hospital Sinathando HIV Clinic, Clairwood Hospital HIV Clinic, King Dinuzulu HIV Clinic, or Wentworth Hospital HIV Clinic). Eligibility criteria were the following: 1) receipt of care and residence within 100 kilometers of a recruitment clinic; 2) age 18 years or older at enrollment; 3) current use of first-line (nonnucleoside reverse transcriptase inhibitor [NNRTI]–based or integrase strand transfer inhibitor [INSTI]–based) ART for at least 5 months; and 4) a plasma viral load of HIV-1 RNA (viral load) of 1000 copies/mL or higher within 90 days of enrollment. We excluded persons with prior known HIV drug resistance, prior exposure to protease inhibitor (PI)–based therapy, or a current indication for change to second-line (PI-based) therapy (such as successive viral load results ≥1000 copies/mL); those with plans to leave the clinic catchment area within the next 9 months; and those who could not provide informed consent.

Viral load result logs were screened to identify potentially eligible participants, who were approached by study staff for informed consent. After completion of informed consent, participants were immediately randomly assigned in a 1:1 ratio in blocks of 4 using the Research Electronic Data Capture (REDCap) tool for study database randomization to the standard of care (SOC) or the GRT strategy of virologic failure management (19). Randomization was stratified by clinic, duration of ART use at enrollment (dichotomized as <1 vs. ≥1 year), and pregnancy status. We initially included use of an INSTI-based regimen as a stratification variable, but no study participants were receiving INSTI-based therapy at the time of enrollment. The randomization sequence was created by the statistician before study initiation and locked within the database such that no other staff members had access.

Study Procedures

Persons in the GRT group were scheduled for the following 3 study visits during the observation period (**Appendix Figure**, available at Annals.org): an enrollment visit, a GRT result interpretation and management visit, and an outcome visit. At the enrollment visit, blood was collected for immediate GRT. We did Sanger sequencing of the reverse transcriptase and protease genes in plasma HIV-1 RNA at the laboratories of the National Health Laboratory Service at Inkosi Albert Luthuli Central Hospital (South Africa) and Joint Clinical Research Centre (Uganda). For specimens that could not be sequenced, a cryopreserved plasma specimen was subsequently tested for viral load to assess whether a low viral load was the cause of failed sequencing. We did GRT interpretation training at all study clinics before study initiation, which included review of HIV genetics, mechanisms of drug resistance, key genetic mutations associated with HIV drug resistance, interpretation of genotypic resistance result reports, and recommendations for regimen selection based on results (the training guide is included in the **Supplement**). Participants were asked to return to the clinic after receipt of resistance testing results for virologic failure management. Clinicians had final decision-making authority for regimen selection. They were advised during the GRT training and in the provided instruction booklets to change to second-line, PI-based therapy if drug resistance patterns suggested resistance to a patient's first-line regimen. Likewise, clinicians were advised to maintain first-line regimens with adherence counseling in the absence of drug resistance or if the viral load had resuppressed in the window between the recruitment viral load measurement and enrollment. Participants in the GRT group returned again 9 months after enrollment for a final study visit for specimen collection and outcome determination.

Participants in the SOC group were scheduled for 4 study visits that corresponded with treatment guidelines for management of virologic failure in South Africa and Uganda, which focus on adherence support for patients with a viral load of 1000 copies/mL or higher. These visits included an enrollment visit, a visit to collect blood for repeated viral load testing, a visit for interpretation and management of viral load results, and a study outcome visit. At the enrollment visit, participants were referred to clinic counseling services on the basis of standardized clinic guidelines for those with virologic failure. They returned for a second visit approximately 2 to 3 months after enrollment so blood could be collected for repeated viral load testing, and for a third visit approximately 1 month later for interpretation of the second viral load result and regimen

selection. On the basis of guidelines from both countries, patients with persistent virologic failure, defined as a repeated viral load of 1000 copies/mL or higher, were recommended to switch to PI-based, second-line therapy, whereas those with a repeated viral load less than 1000 copies/mL were recommended to continue receiving first-line therapy. Similar to the GRT group, participants in the SOC group were scheduled for a final visit approximately 9 months after enrollment for specimen collection and outcome determination. In both groups, clinical care was provided at the clinics by clinic staff per routine clinical protocols in the periods between study visits.

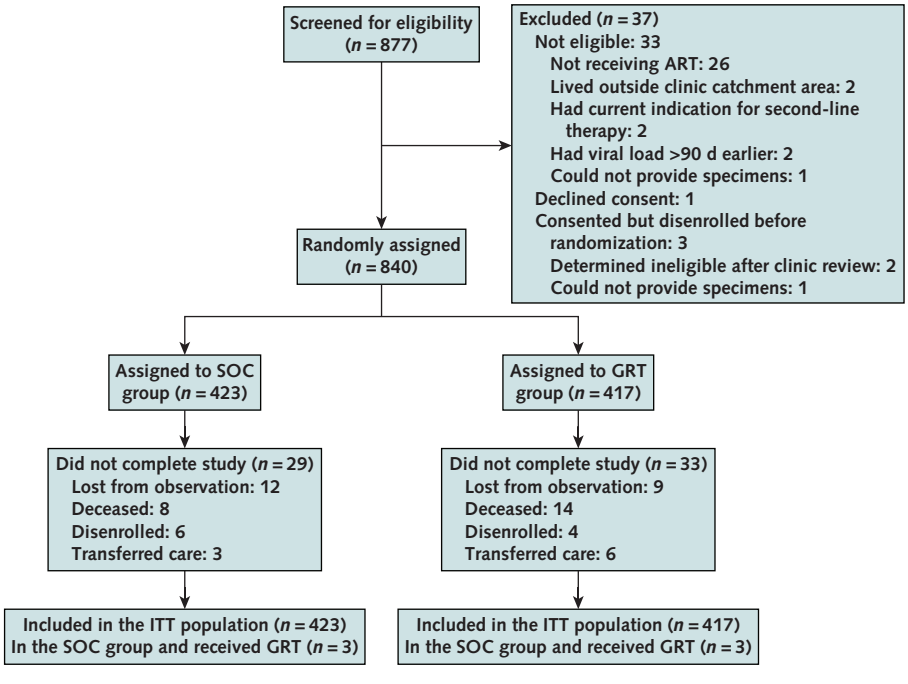
Outcome Measures

The prespecified primary outcome was achievement of a viral load below 200 copies/mL at the 9-month outcome visit. Viral load results falling in a window from 2 weeks before through 12 weeks after this visit were eligible for assessment. We defined a successful outcome in participants with a viral load less than 200 copies/mL during the window and a failed outcome in participants with a viral load of 200 copies/mL or greater or with missing viral load data during the window period. The study underwent a single major protocol change in 2020, when the study window was extended for participants who were actively enrolled in the trial but unable to return to the clinic for their outcome assessment while COVID-19 transportation restrictions were imposed in both countries (24 March 2020 in Uganda and 27 March 2020 in South Africa; details of the protocol change are included in the Study Analysis Plan section of the Supplement). Prespecified secondary outcomes were the proportion of participants with an undetectable viral load (below limit of detection) at study conclusion; the proportion of participants with an undetectable viral load receiving first-line (NNRTI-based) therapy at study conclusion; the proportion of participants with HIV drug resistance mutations (as defined by the International Antiviral Society–USA) to their regimen at study conclusion (20), determined by GRT, which was done for all participants with a viral load of 1000 copies/mL or higher at study conclusion; retention in HIV clinical care through study completion; and 9-month survival. Resource use, costs, and utility data were also collected and are being analyzed as part of an accompanying cost-effectiveness analysis.

Statistical Analysis

The study was powered to detect a between-group difference of 10 percentage points or more in the proportion of participants with a viral load less than 200 copies/mL at the 9-month outcome visit. We selected this difference on the basis of consultation with in-country partners as a clinically meaningful benefit to result in changes to care guidelines. On the basis of published resuppression rates after first-line treatment failure in sub-Saharan Africa (21–24), we estimated that approximately 68% of participants in the SOC group would remain in care and achieve resuppression. Because treatment was considered to have failed in participants with missing viral load data, all patients were included in the analysis of the primary outcome; there was no reduction in analytic sample size due to

Figure 1. Study flow diagram.



ART = antiretroviral therapy; GRT = genotypic resistance testing; ITT = intention-to-treat; SOC = standard of care.

Table. Demographic and Clinical Characteristics of Study Participants at Enrollment

Characteristic	Total Cohort (*n* = 840)	Standard of Care Group (*n* = 423)	Genotypic Resistance Testing Group (*n* = 417)
Sex, *n (%)*			
Female	430 (51)	221 (53)	209 (50)
Male	410 (49)	202 (48)	208 (50)
Pregnancy, *n (%)*			
Pregnant at enrollment	13 (2)	7 (2)	6 (1)
Not pregnant at enrollment	827 (98)	416 (98)	411 (99)
Median age (IQR), *y*	37 (31–45)	37 (31–45)	37 (30–44)
Country, *n (%)*			
Uganda	420 (50)	210 (50)	210 (50)
South Africa	420 (50)	213 (50)	207 (50)
Median ART duration (IQR), *y*	3.2 (1.1–6.4)	3.5 (1.1–6.5)	3.0 (1.1–6.4)
Median CD4 count (IQR), × 10^9 *cells/L*	0.281 (0.121–0.457)	0.303 (0.132–0.475)	0.259 (0.112–0.434)
ART regimen, *n (%)*			
TDF–3(F)TC–EFV	606 (72)	311 (75)	295 (71)
AZT–3TC–NVP	118 (14)	60 (14)	58 (14)
Other	116 (14)	52 (12)	64 (15)
Self-reported treatment adherence at enrollment, *n (%)*			
<100% in prior 30 d	592 (70)	297 (70)	295 (71)
100% in prior 30 d	248 (30)	126 (30)	122 (29)

3(F)TC = lamivudine or emtricitabine; ART = antiretroviral therapy; AZT = zidovudine; EFV = efavirenz; IQR = interquartile range; NVP = nevirapine; TDF = tenofovir.

losses from observation. Consequently, we enrolled 420 participants in each group for a total sample of 840 participants to give us 88% power to identify a 10–percentage point difference in the primary outcome measure between groups, allowing for a type I error rate of 5% with a superiority design.

We first summarized demographic and clinical characteristics of the cohort by study group. The primary efficacy analysis was done on an intention-to-treat basis according to initial randomization allocation and regardless of completion of GRT. To test for the difference in primary and secondary outcomes between the study groups, we fitted stratified conditional logistic regression models for each outcome of interest with treatment group as the explanatory variable of interest and clinic, duration of ART use, and pregnancy status as strata. To report the overall proportion of participants who achieved each outcome, we computed a weighted average of sample proportions with weight equal to the stratum-level frequency divided by the total sample size; SEs were computed accordingly. Although we had initially planned an as-treated analysis for participants who experienced crossover (that is, participants in the GRT group who did not complete GRT and those in the SOC group who did complete GRT), this occurred in only 3 participants, so we did not proceed with this analysis. We also used conditional logistic regression models to estimate the effect of resistance testing on achieving a viral load less than 200 copies/mL in prespecified analyses in the following participant subgroups: CD4 count less than versus greater than or equal to 0.200×10^9 cells/L at enrollment, men versus women, self-reported adherence at enrollment of less than 100% versus 100%, and enrollment in Uganda versus South Africa. We did a post hoc sensitivity analysis in which we excluded persons who had a viral load measurement at an out-of-window outcome visit after the COVID-19 restrictions began. Finally, in post hoc analyses, we fitted mixed-effects logistic regression models of our primary outcome of interest to assess for residual confounding, applying fixed effects for study group, sex, age at enrollment (>35 vs. ≤35 years), CD4 T-cell count at enrollment, viral load at enrollment (>10 000 vs. ≤10 000 copies/mL), and country, as well as a random effect for strata. Analyses were done in duplicate by 2 study statisticians (B.A.J. and N.M.) using analysis packages in R, version 4.1.0 (R Foundation), and Stata, version 15 (StataCorp).

Role of the Funding Source

The President's Emergency Plan for AIDS Relief and the National Institute of Allergy and Infectious Diseases had no role in the design or conduct of the study, the analysis or interpretation of the data, the drafting of the manuscript, or the decision to submit the results for publication.

RESULTS

Participants

Between December 2016 and December 2019, a total of 877 persons were screened for eligibility and 840 (96%) were randomly assigned to a group, 417 to the GRT group and 423 to the SOC group (**Figure 1**). Enrollment was evenly distributed between Uganda (*n* =

420 [50%]) and South Africa (*n* = 420 [50%]), and approximately half of enrolled participants were women (*n* = 430 [51%]) (**Table**). The median age was 37 years (interquartile range, 31 to 45 years), the median duration of ART use was 3.2 years (interquartile range, 1.1 to 6.4 years), the median CD4 T-cell count at enrollment was 0.281×10^9 cells/L (interquartile range, 0.121 to 0.456×10^9 cells/L), and 606 participants (72%) were receiving a regimen of tenofovir disoproxil fumarate, emtricitabine or lamivudine, and efavirenz.

Study Flow and Adherence to Guidelines

All participants were enrolled before the COVID-19 epidemic restrictions began, but 170 of 840 (21%) had a final study visit scheduled after the restrictions began. The study groups did not differ in the proportion of participants affected by the COVID-19 restrictions (87 of 423 [20.6%] in the SOC group vs. 83 of 417 [19.9%] in the GRT group). Of these 170 participants, 47 (27.6%) completed an outcome visit blood collection outside the prespecified window, including 5.0% (21 of 423) in the SOC group and 6.2% (26 of 417) in the GRT group. In the total cohort, 778 participants (93%; 394 of 423 [93%] in the SOC group and 384 of 417 [92%] in the GRT group) completed their 9-month outcome visit, whereas 22 (3%) died, 21 (3%) were lost from observation, 10 (1%) disenrolled, and 9 (1%) moved out of the catchment area during the study. Rates of study retention and completion were similar in the pre–COVID-19 period (622 of 770 [93%]) and the COVID-19 period (157 of 170 [92%]).

All 417 persons in the GRT group (100%) had GRT completed at study enrollment (**Figure 2**). Of these, 282 (67.6%) had detectable drug resistance and 135 (32.4%) had either absence of drug resistance or a viral load too low to allow successful sequencing. Of those with detectable drug resistance, 243 of 282 (86.2%) changed from first-line to second-line therapy during the study at a clinician's direction, 15 (5.3%) continued to receive first-line therapy, and 24 (8.5%) dropped out before a regimen decision was made. Of those without drug resistance detected, 95 of 135 (70.4%) continued to receive first-line therapy throughout the study, 31 (23.0%) changed to second-line therapy, and 9 (6.7%) dropped out before a regimen decision was made.

In the SOC group, 396 of 423 participants (93%) completed a repeated viral load test during the study. On repeated testing, approximately 38% of participants in this group (162 to 423) had a viral load lower than 1000 copies/mL and 234 of 423 (55.3%) had a viral load of 1000 copies/mL or higher. Of those with a repeated viral load measurement lower than 1000 copies/mL, 129 (79.6%) continued to receive first-line therapy, 27 (16.7%) changed to second-line therapy, and 6 (3.7%) dropped out before a regimen change. Finally, of those in the SOC group with a repeated viral load measurement of 1000 copies/mL or higher or no repeated viral load measurement during the study (*n* = 261), 210 (80.4%) changed to second-line therapy, 28 (10.7%) continued to receive first-line therapy, and 23 (8.8%) dropped out before a regimen change.

Primary Outcome

Rates of achievement of a viral load below 200 copies/mL were similar 9 months after enrollment in the GRT

Figure 2. Progress through the study, by group.

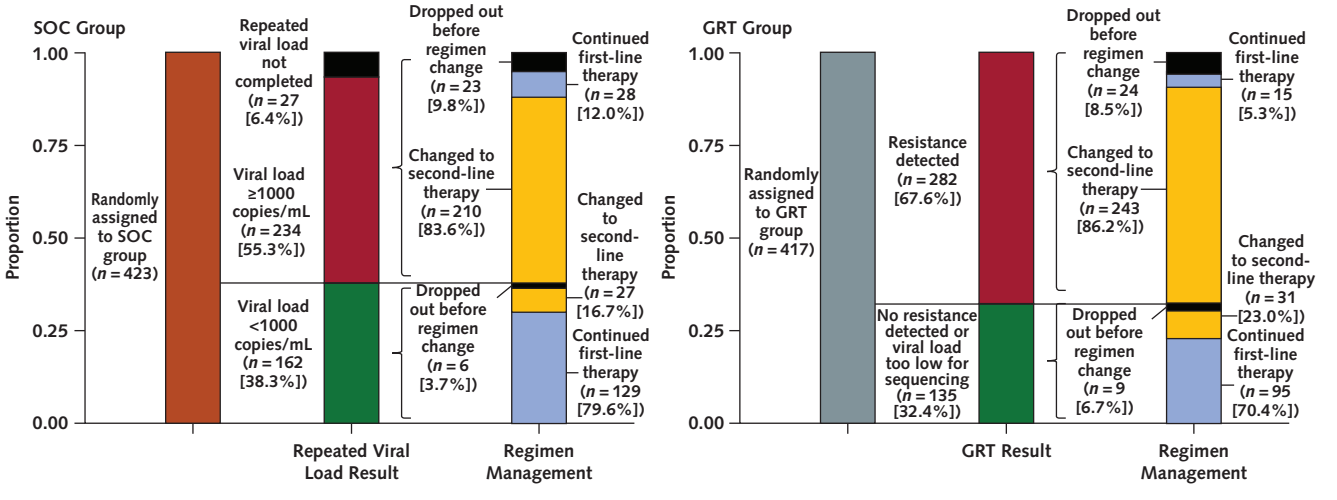
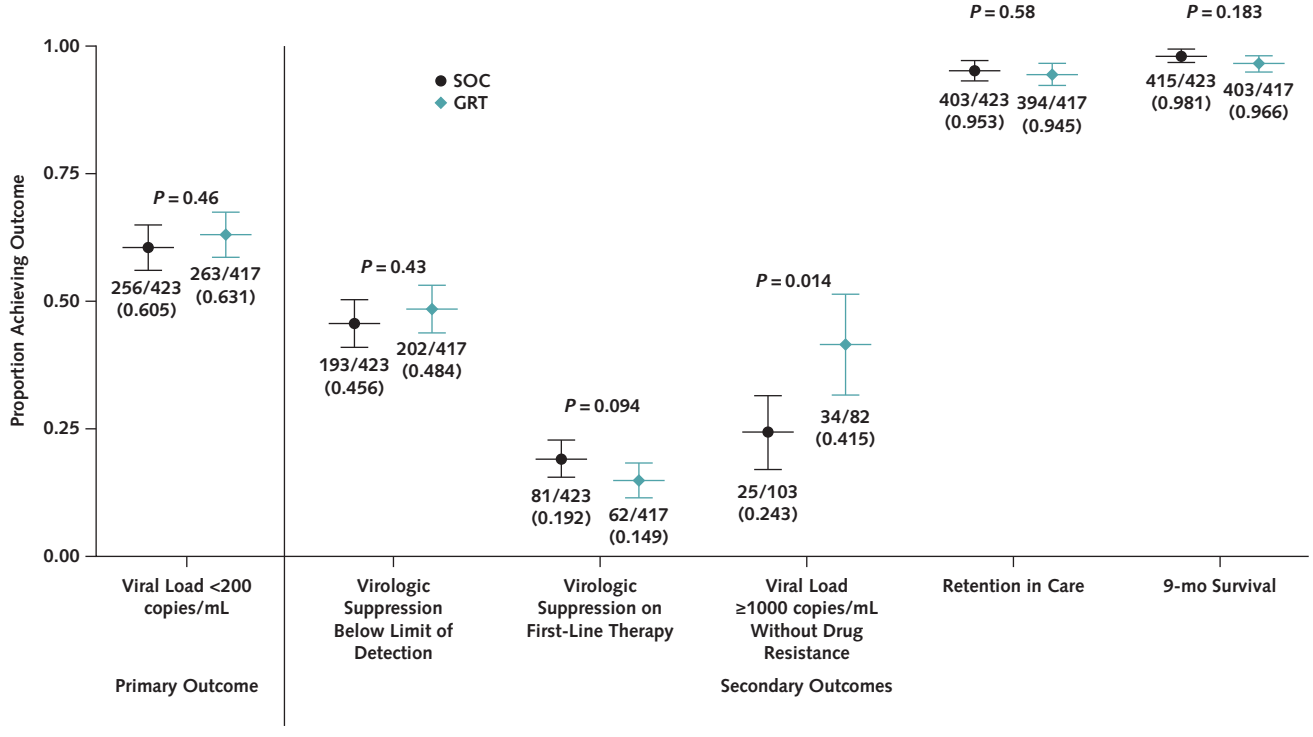


Diagram showing progress through the study from enrollment through completion of recommended laboratory testing and results reporting in the SOC group (*left*) and GRT group (*right*). The figures move from left to right across the study timeline from enrollment and randomization to completion of a repeated viral load test after adherence counseling and viral load results reporting in the SOC group, or from results of the initial resistance test and results reporting in the resistance testing group. The red bars signal high viral loads or the presence of genotypic resistance, whereas the green bars signify low viral loads or the absence of genotypic resistance. In the final column, light blue bars represent participants who continued to receive first-line therapy, whereas yellow bars represent those who changed to second-line therapy. Black bars signal participants who were lost to follow-up or died. GRT = genotypic resistance testing; SOC = standard of care.

Figure 3. Primary and secondary outcomes, by study group.



Proportion of participants achieving primary and secondary outcomes in the SOC (*black boxes*) and GRT (*green boxes*) groups. Error bars represent 95% CIs and were calculated using stratum-level frequency weighting, analogous to estimation of the Cochran–Mantel–Haenszel test statistic. *P* values were estimated using conditional logistic regression with each outcome as the dependent variable, group as the dependent variable, and strata as matching variables. GRT = genotypic resistance testing; SOC = standard of care.

and SOC groups (263 of 417 [63%] vs. 256 of 423 [61%]; odds ratio [OR], 1.11 [95% CI, 0.83 to 1.49]) (Figure 3). Results were similar in subgroup analyses stratified by CD4 T-cell count at enrollment, self-reported adherence at enrollment, and country (Figure 4). Results were also similar after exclusion of participants with blood drawn at an out-of-window outcome visit that occurred after the COVID-19 restrictions began (OR, 1.10 [CI, 0.82 to 1.49]). However, we did find a modest difference in the effect of GRT on virologic suppression by sex (OR, 0.85 [CI, 0.56 to 1.28] among women vs. 1.37 [CI, 0.91 to 2.08] among men). Resuppression rates for those in the GRT group with and without resistance at enrollment were 167 of 282 (59%) and 96 of 135 (71%), respectively, whereas in the SOC group, resuppression rates for those with a repeated viral load measurement less than 1000 copies/mL and greater than or equal to 1000 copies/mL were 116 of 162 (72%) and 127 of 234 (54%), respectively. Finally, in a mixed-effects multivariable regression model adjusted for potential confounders, the effect of resistance testing remained similar (adjusted OR, 1.18 [CI, 0.87 to 1.60]), but country of enrollment (adjusted OR, 0.27 [CI, 0.19 to 0.37] for South Africa vs. Uganda) and age over 35 years (adjusted OR, 1.57 [CI, 1.15 to 2.16]) were each associated with achievement of virologic suppression less than 200 copies/mL 9 months later (Appendix Table, available at Annals.org).

Secondary Outcomes

We found no difference between the GRT and SOC groups in the odds of virologic suppression less than the limit of assay detection (202 of 417 [48%] vs. 193 of 423 [46%]; OR, 1.12 [CI, 0.85 to 1.47]), in the odds of achieving viral suppression at 9 months on a first-line regimen (62 of 417 [15%] vs. 81 of 423 [19%]; OR, 0.73 [CI, 0.51 to 1.06]), in the odds of 9-month retention in care (394 of 417 [94%] vs. 403 of 423 [95%]; OR, 0.84 [CI, 0.45 to 1.56]), or in the odds of 9-month survival (403 of 417 [97%] vs. 415 of 423 [98%]; OR, 0.55 [CI, 0.23 to 1.33]) (Figure 3). By contrast, among participants who had a viral load of 1000 copies/mL or higher at 9 months, those in the GRT group were more likely to have treatment failure without drug resistance to their ART regimen (34 of 82 [41%] vs. 25 of 103 [24%]; OR, 2.30 [CI, 1.22 to 4.35]).

Discussion

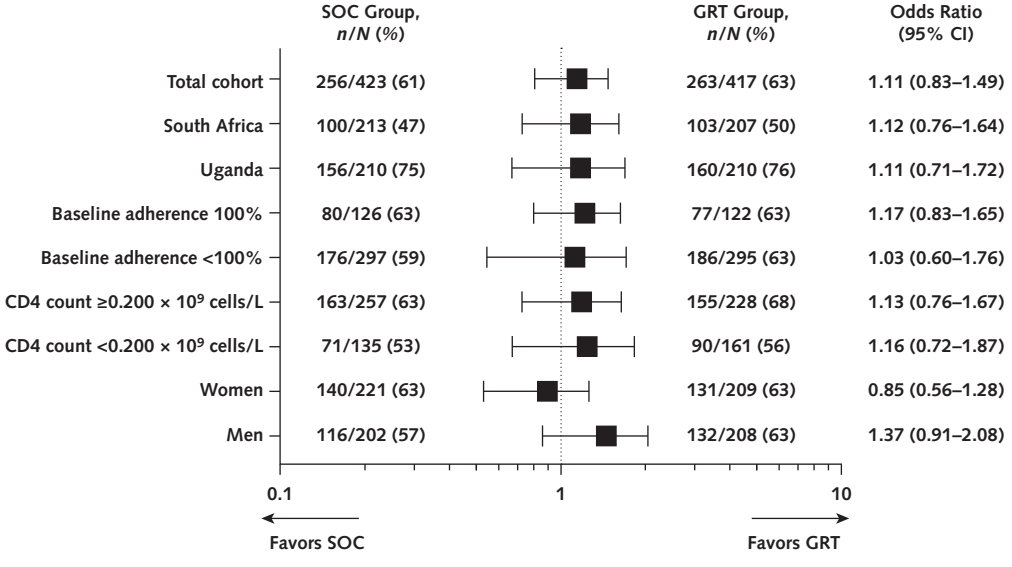
Contrary to our hypothesis, we found that the addition of GRT to routine care for persons whose first-line ART failed in public-sector HIV clinics in Uganda and South Africa was not associated with improved 9-month rates of

virologic suppression. Of note, only approximately 60% of participants in both groups achieved virologic resuppression during the observation period (75% in Uganda and 48% in South Africa), despite access to either virologic monitoring or GRT, as well as adherence counseling support and access to PI-based second-line regimens. These resuppression rates are 5 to 30 percentage points lower than results from prior clinical trials that estimated the efficacy of second-line regimens after first-line failure in the same region (25–28). Unlike many of these prior studies, our study was done fully within the public sector, including use of clinical staff and laboratory support infrastructures. Our results reinforce the critical need for and persistent challenge of finding effective interventions for persons who have virologic failure after ART initiation in the public sector in sub-Saharan Africa. As treatment eligibility and access expand, this final step in the virologic cascade will increasingly be responsible for HIV-related morbidity, mortality, and transmission (29, 30). Our findings are of particular importance in such countries as Uganda and South Africa, where virologic suppression is recommended before switching from NNRTI-based to newer INSTI-based first-line therapy, as well as for selected populations who retain an indication for NNRTI-based therapy. Moreover, our low resuppression rates for those with wild-type virus accentuate the notion that higher-potency regimens alone are not likely to solve the challenge of persistent virologic failure in the region.

Previous randomized controlled trials of resistance testing after virologic failure in the United States and Europe by and large had similar results. Namely, they found that patients receiving GRT had more active drugs at study conclusion, but only 1 of 3 studies reported improved virologic suppression rates at the end of the observation period (9–11). Those studies used a similar open design but were done in the early years of ART access, variously considered genotypic and phenotypic resistance testing, were smaller, and tended to include participants with multiclass drug resistance. Because our study was the first to our knowledge to assess the benefit of GRT after first-line failure in sub-Saharan Africa, and in light of this prior body of data, our results do not support implementation of routine GRT among patients in sub-Saharan Africa whose first-line therapy fails.

Our study also had some secondary findings of relevance. First, among participants with a viral load of 1000 copies/mL or higher, we identified substantially increased odds (OR, 2.30 [CI, 1.22 to 4.35]) of having resistance to their regimen at the conclusion of the study in the SOC group compared with the GRT group. Although not as consequential as achievement of virologic resuppression, this magnitude of reduction in drug resistance with persistent failure might have important secondary benefits for both patients and society by preserving treatment options for the future. Forthcoming cost-effectiveness analyses will better elucidate the broader consequences of this finding for populations and health systems (31). Second, we found preliminary evidence in support of GRT after virologic failure for men (OR, 1.37 [CI, 0.91 to 2.08]) but not women (OR, 0.85 [CI, 0.56 to 1.28]). Although a prespecified analysis, our study was not powered to detect differences within subgroups. Thus, this finding warrants further exploration, particularly given the evident need for interventions that improve virologic suppression rates among men in the region (32–34). Finally, although allocation to GRT did not predict virologic resuppression, some characteristics at enrollment did, including enrollment in Uganda versus South Africa, higher self-reported

Figure 4. Effect of GRT on virologic resuppression in total cohort and subgroups.



Forest plot showing the association between the study intervention (GRT) and primary outcomes, defined as achievement of a viral load of <200 copies/mL 9 mo after enrollment. Results are shown for the overall cohort and prespecified subgroups. Odds ratios were estimated from conditional logistic regression models, which include randomization strata in the model structure. GRT = genotypic resistance testing; SOC = standard of care.

adherence, and a CD4 count greater than or equal to 0.200×10^9 cells/L—each of which suggest that differential models of care could be used to identify persons with high risk for persistent treatment failure for receipt of additional services (35).

A primary limitation of our study was the focus on persons whose first-line NNRTI-based therapy failed; this focus occurred because enrollment was completed before programmatic transitions to INSTI-based first-line therapy, and with lopinavir–ritonavir and atazanavir–ritonavir as the predominant second-line therapies. Additional studies will be needed to extrapolate our results to persons in whom newer treatment regimens (which may confer higher barriers to resistance and greater tolerability) fail. Nonetheless, and as previously discussed, our findings remain relevant for large populations of persons who continue to receive NNRTI-based therapy worldwide, for those whose treatment fails with wild-type virus (because the determinants of and solutions to virologic failure due to adherence challenges are unlikely to be resolved simply by selecting drugs with higher barriers to resistance), and as a general indication of the challenges of addressing virologic failure in the public sector. This study also had an open design, which is typically unavoidable for studies that assess the effect of diagnostic tests on patient care. We provided genotypic resistance training at the participating clinics and monitored whether participants were transitioned through care on the basis of guidelines and resistance testing results, which occurred in 87% of participants (676 of 778) who remained in care through the study. The imperfect rate of guideline adherence was likely due to the conduct of our study in the public sector, such that treatment decisions were left to the discretion of clinic providers. Thus, our results likely better reflect real-world implementation of GRT than a scenario in which testing results are strictly adhered to for regimen decisions. We measured our primary outcome 9 months after enrollment, so our results cannot indicate whether resistance testing affects outcomes over a longer time horizon. Finally, these data should be interpreted in light of our study population, which included persons in care in semiurban and urban HIV clinics in the public sector in South Africa and Uganda. These high-volume clinics are reflective of the pressures and challenges of HIV care in the public sector across much of the region, but findings from these clinics may not be generalizable to smaller or private-sector settings.

In conclusion, the addition of GRT to routine care did not improve virologic outcomes for patients in whom first-line ART failed in public-sector HIV clinics in South Africa and Uganda. Interventions that improve virologic outcomes for this high-risk population remain elusive and a priority to ensure that global goals for HIV control are achieved.

From Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts, Mbarara University of Science and Technology, Mbarara, Uganda, Africa Health Research Institute, KwaZulu-Natal, South Africa, and University of KwaZulu-Natal, Durban, South Africa (M.J.S.); University of KwaZulu-Natal, Durban, South Africa (M.S.M., S.P., J.B., G.G., H.S.); Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts (S.M., K.A., R.T.G.); Massachusetts General Hospital, Boston, Massachusetts (R.F.G.); Mbarara University of Science and Technology, Mbarara, Uganda (I.A., W.M., N.M., G.M., M.B.B.); National Health Laboratory Service, Durban, South Africa (M.P., P.M.); Griffith University, Brisbane, Queensland, Australia (T.R.); University of Rochester, Rochester, New York (B.A.J.); and Emory University School of Medicine and Rollins School of Public Health, Atlanta, Georgia (V.C.M.).

Note: The study investigators designed and oversaw the study and are responsible for the data quality and completeness, data analysis, and adherence to the study protocol.

Acknowledgment: The authors thank the following study, laboratory, and clinical staff for their tireless commitment and support of this study: Ntwenhle Makhaza, Nikiwe Ntanzi, Crisanne Dhevar, Sultana Abdool, Ansuri Singh, Dr. Eseza Monica Okunga Nambassi, Sabelo DlaDla, Agumenaitwe Patrick, Tumwesigyire Jonan, Kusingura Caroline, Komujuni Adah, Baryahikwa Hassan, and Agaba Edson. They also thank the study participants for their interest, partnership, and contributions to the REVAMP study.

Grant Support: By grant NIH R01 AI124718 from the President's Emergency Plan for AIDS Relief and the National Institute of Allergy and Infectious Diseases of the National Institutes of Health. Dr. Marconi also received support from the National Institute of Allergy and Infectious Diseases (R01AI098558-01A1 and R01AI098558-04S1) and the Emory Center for AIDS Research (P30AI050409). Dr. Gandhi also received support from the Harvard University Center for AIDS Research (NIH P30 AI060354) and the AIDS Clinical Trials Group (NIH/NIAID 2 UMAI069412-09).

Disclosures: Disclosures can be viewed at www.acponline.org/authors/icmje/ConflictOfInterestForms.do?msNum=M21-2229.

Data Sharing Statement: The following data will be made available: deidentified analytic data set (data requests can be made to the corresponding author at msiedner@mgh.harvard.edu). The following supporting documents are available in the Supplement: study protocol, study analysis plan, analytic code, and output. These data will be made available to researchers whose proposed use of the data has been approved, for any purpose; data will be made available on request to the corresponding author. Requests for investigator or analytic support will also be considered (restrictions: none).

Corresponding Author: Mark J. Siedner, MD, Medical Practice Evaluation Center, Massachusetts General Hospital, 100 Cambridge Street, Suite 1600, Boston, MA 02114; e-mail, msiedner@mgh.harvard.edu.

Current author addresses and author contributions are available at Annals.org.

References

1. **Gupta-Wright A, Fielding K, van Oosterhout JJ, et al.** Virological failure, HIV-1 drug resistance, and early mortality in adults admitted to hospital in Malawi: an observational cohort study. Lancet

HIV. 2020;7:e620-e628. [PMID: 32890497] doi:10.1016/S2352-3018(20)30172-7
2. **Petersen ML, Tran L, Geng EH, et al.** Delayed switch of antiretroviral therapy after virologic failure associated with elevated mortality among HIV-infected adults in Africa. AIDS. 2014;28:2097-107. [PMID: 24977440]
3. **Palombi L, Marazzi MC, Guidotti G, et al; DREAM Program.** Incidence and predictors of death, retention, and switch to second-line regimens in antiretroviral-treated patients in sub-Saharan African sites with comprehensive monitoring availability. Clin Infect Dis. 2009;48:115-122. [PMID: 20380075]
4. **Attia S, Egger M, Müller M, et al.** Sexual transmission of HIV according to viral load and antiretroviral therapy: systematic review and meta-analysis. AIDS. 2009;23:1397-404. [PMID: 19381076] doi:10.1097/QAD.0b013e32832b7dca
5. **Quinn TC, Wawer MJ, Sewankambo N, et al; Rakai Project Study Group.** Viral load and heterosexual transmission of human immunodeficiency virus type 1. N Engl J Med. 2000;342:921-9. [PMID: 10738050]
6. **Grabowski MK, Patel EU, Nakigozi G, et al.** Prevalence and predictors of persistent human immunodeficiency virus viremia and viral rebound after universal test and treat: a population-based study. J Infect Dis. 2021;223:1150-1160. [PMID: 33471100] doi:10.1093/infdis/jiab021
7. **Panel on Antiretroviral Guidelines for Adults and Adolescents.** Guidelines for the Use of Antiretroviral Agents in Adults and Adolescents with HIV. Department of Health and Human Services. Updated 18 December 2019. Accessed at https://clinicalinfo.hiv.gov/sites/default/files/guidelines/documents/AdultandAdolescentGL.pdf on 9 March 2021.
8. **Durant J, Clevenbergh P, Halfon P, et al.** Drug-resistance genotyping in HIV-1 therapy: the VIRADAPT randomised controlled trial. Lancet. 1999;353:2195-9. [PMID: 10392984]
9. **Cohen CJ, Hunt S, Sension M, et al; VIRA3001 Study Team.** A randomized trial assessing the impact of phenotypic resistance testing on antiretroviral therapy. AIDS. 2002;16:579-88. [PMID: 11873001]
10. **Tural C, Ruiz L, Holtzer C, et al; Havana Study Group.** Clinical utility of HIV-1 genotyping and expert advice: the Havana trial. AIDS. 2002;16:209-18. [PMID: 11807305]
11. **Haubrich RH, Kemper CA, Hellmann NS, et al; California Collaborative Treatment Group.** A randomized, prospective study of phenotype susceptibility testing versus standard of care to manage antiretroviral therapy: CCTG 575. AIDS. 2005;19:295-302. [PMID: 15718840]
12. **World Health Organization.** Updated recommendations on first-line and second-line antiretroviral regimens and post-exposure prophylaxis and recommendations on early infant diagnosis of HIV. 1 January 2018. Accessed at www.who.int/publications/i/item/WHO-CDS-HIV-18.51 on 15 February 2021.
13. **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;107:28-30. [PMID: 28112085] doi:10.7196/SAMJ.2016.v107.i1.12058
14. **Inzaule SC, Ondoa P, Peter T, et al.** Affordable HIV drug-resistance testing for monitoring of antiretroviral therapy in sub-Saharan Africa. Lancet Infect Dis. 2016;16:e267-e275. [PMID: 27569762] doi:10.1016/S1473-3099(16)30118-9
15. **Iwuji CC, Shahmanesh M, Koole O, et al; H-DREAM Network.** Clinical outcomes after first-line HIV treatment failure in South Africa: the next cascade of care. HIV Med. 2020;21:457-462. [PMID: 32495515] doi:10.1111/hiv.12877
16. **Glass TR, Motaboli L, Nsakala B, et al.** The viral load monitoring cascade in a resource-limited setting: a prospective multicentre cohort study after introduction of routine viral load monitoring in rural Lesotho. PLoS One. 2019;14:e0220337. [PMID: 31461455] doi:10.1371/journal.pone.0220337
17. **Ssempijja V, Nakigozi G, Chang L, et al.** Rates of switching to second-line antiretroviral therapy and impact of delayed switching on immunologic, virologic, and mortality outcomes among HIV-infected adults with virologic failure in Rakai, Uganda. BMC Infect Dis. 2017;17:582. [PMID: 28830382] doi:10.1186/s12879-017-2680-6
18. **Siedner MJ, Bwana MB, Moosa MS, et al.** The REVAMP trial to evaluate HIV resistance testing in sub-Saharan Africa: a case study in clinical trial design in resource limited settings to optimize effectiveness and cost effectiveness estimates. HIV Clin Trials. 2017;18:149-155. [PMID: 28720039] doi:10.1080/15284336.2017.1349028
19. **Harris PA, Taylor R, Minor BL, et al; REDCap Consortium.** The REDCap consortium: building an international community of software platform partners. J Biomed Inform. 2019;95:103208. [PMID: 31078660] doi:10.1016/j.jbi.2019.103208
20. **Wensing AM, Calvez V, Ceccherini-Silberstein F, et al.** 2019 update of the drug resistance mutations in HIV-1. Top Antivir Med. 2019;27:111-121. [PMID: 31634862]
21. **Hoffmann CJ, Charalambous S, Sim J, et al.** Viremia, resuppression, and time to resistance in human immunodeficiency virus (HIV) subtype C during first-line antiretroviral therapy in South Africa. Clin Infect Dis. 2009;49:1928-35. [PMID: 19911963] doi:10.1086/648444
22. **El-Khatib Z, Delong AK, Katzenstein D, et al.** Drug resistance patterns and virus re-suppression among HIV-1 subtype C infected patients receiving non-nucleoside reverse transcriptase inhibitors in South Africa. J AIDS Clin Res. 2011;2. [PMID: 21927716]
23. **Jobanputra K, Parker LA, Azih C, et al.** Factors associated with virological failure and suppression after enhanced adherence counselling, in children, adolescents and adults on antiretroviral therapy for HIV in Swaziland. PLoS One. 2015;10:e0116144. [PMID: 25695494] doi:10.1371/journal.pone.0116144
24. **Ciaffi L, Koulla-Shiro S, Sawadogo A, et al; 2LADY Study Group.** Efficacy and safety of three second-line antiretroviral regimens in HIV-infected patients in Africa. AIDS. 2015;29:1473-81. [PMID: 26244387] doi:10.1097/QAD.0000000000000709
25. **Aboud M, Kaplan R, Lombaard J, et al.** Dolutegravir versus ritonavir-boosted lopinavir both with dual nucleoside reverse transcriptase inhibitor therapy in adults with HIV-1 infection in whom first-line therapy has failed (DAWNING): an open-label, non-inferiority, phase 3b trial. Lancet Infect Dis. 2019;19:253-264. [PMID: 30732940] doi:10.1016/S1473-3099(19)30036-2
26. **Boyd MA, Kumarasamy N, Moore CL, et al; SECOND-LINE Study Group.** Ritonavir-boosted lopinavir plus nucleoside or nucleotide reverse transcriptase inhibitors versus ritonavir-boosted lopinavir plus raltegravir for treatment of HIV-1 infection in adults with virological failure of a standard first-line ART regimen (SECOND-LINE): a randomised, open-label, non-inferiority study. Lancet. 2013;381:2091-9. [PMID: 23769235] doi:10.1016/S0140-6736(13)61164-2
27. **Hakim JG, Thompson J, Kityo C, et al; Europe Africa Research Network for Evaluation of Second-line Therapy (EARNEST) Trial Team.** Lopinavir plus nucleoside reverse-transcriptase inhibitors, lopinavir plus raltegravir, or lopinavir monotherapy for second-line treatment of HIV (EARNEST): 144-week follow-up results from a randomised controlled trial. Lancet Infect Dis. 2018;18:47-57. [PMID: 29108797] doi:10.1016/S1473-3099(17)30630-8
28. **Paton NI, Musaazi J, Kityo C, et al; NADIA Trial Team.** Dolutegravir or darunavir in combination with zidovudine or tenofovir to treat HIV. N Engl J Med. 2021;385:330-341. [PMID: 34289276] doi:10.1056/NEJMoa2101609
29. **Nachega JB, Sam-Agudu NA, Mofenson LM, et al.** Achieving viral suppression in 90% of people living with human immunodeficiency virus on antiretroviral therapy in low- and middle-income countries: progress, challenges, and opportunities. Clin Infect Dis. 2018;66:1487-1491. [PMID: 29324994] doi:10.1093/cid/ciy008

30. **Vandormael A, Akullian A, Siedner M, et al.** Declines in HIV incidence among men and women in a South African population-based cohort. Nat Commun. 2019;10:5482. [PMID: 31792217] doi:10.1038/s41467-019-13473-y
31. **Rautenberg TA, George G, Bwana MB, et al.** Comparative analyses of published cost effectiveness models highlight critical considerations which are useful to inform development of new models. J Med Econ. 2020;23:221-227. [PMID: 31835974] doi:10.1080/13696998.2019.1705314
32. **Hermans LE, Carmona S, Nijhuis M, et al.** Virological suppression and clinical management in response to viremia in South African HIV treatment program: a multicenter cohort study. PLoS Med. 2020;17:e1003037. [PMID: 32097428] doi:10.1371/journal.pmed.1003037
33. **Kerkhoff AD, Sikombe K, Eshun-Wilson I, et al.** Mortality estimates by age and sex among persons living with HIV after ART initiation in Zambia using electronic medical records supplemented with tracing a sample of lost patients: a cohort study. PLoS Med. 2020;17:e1003107. [PMID: 32401797] doi:10.1371/journal.pmed.1003107
34. **Tsai AC, Siedner MJ.** The missing men: HIV treatment scale-up and life expectancy in sub-Saharan Africa. PLoS Med. 2015;12:e1001906. [PMID: 26599825] doi:10.1371/journal.pmed.1001906
35. **Grimsrud A, Bygrave H, Doherty M, et al.** Reimagining HIV service delivery: the role of differentiated care from prevention to suppression. J Int AIDS Soc. 2016;19:21484. [PMID: 27914186] doi:10.7448/IAS.19.1.21484

Current Author Addresses: Dr. Siedner and Ms. Gilbert: Medical Practice Evaluation Center, Massachusetts General Hospital, 100 Cambridge Street, Suite 1600, Boston, MA 02114.
Dr. Moosa: Department of Infectious Diseases, Division of Internal Medicine, Room Z319, 3rd Floor, Nelson Mandela School of Medicine, 719 Umbilo Road, Congella, 4013, KZN, South Africa.
Dr. McCluskey: 100 Cambridge Street, Suite 1600, Boston, MA 02114.
Mr. S. Pillay: 111 Granada Street, Shallcross, 4093, South Africa.
Mr. Aturinda: Mbarara University Grants Office, Box 1410, Mbarara, Uganda.
Drs. Ard and Gandhi: Massachusetts General Hospital, 55 Fruit Street, GRJ-504, Boston, MA 02114.
Dr. Muyindike: Mbarara University of Science and Technology, Mbarara-Kabale High way, PO Box 1410, Mbarara, Uganda.
Mr. Musinguzi: Plot 10/24, Lower Circular Road, Mbarara, Uganda.
Mr. Masette: Mbarara University of Science and Technology, MGO Office, PO Box 1410, Mbarara, Uganda.
Mr. M. Pillay and Dr. Moodley: 800 Vusi Mzimela Road, Inkosi Albert Luthuli Central Hospital, Umkabaan (Cato Manor), Durban, 4091, South Africa.
Dr. Brijkumar: 157 Hillhead Road, Brighton Beach Bluff, Durban, 4052, South Africa.
Dr. Rautenberg: N78, Level 2, Room 2.34 Centre for Applied Health Economics, Griffith University Nathan Campus, 170 Kessels Road, Nathan, QLD 4111, Australia.
Dr. George: University of KwaZulu-Natal, Westville Campus, J Block 4th Floor, Durban, 4041, South Africa.
Dr. Johnson: 601 Elmwood Avenue, Box 630, Rochester, NY 14642.
Dr. Sunpath: PO Box 70820, Overport, 4067, South Africa.
Dr. Marconi: 1760 Haygood Drive NE, Room W325, Atlanta, GA 30322.

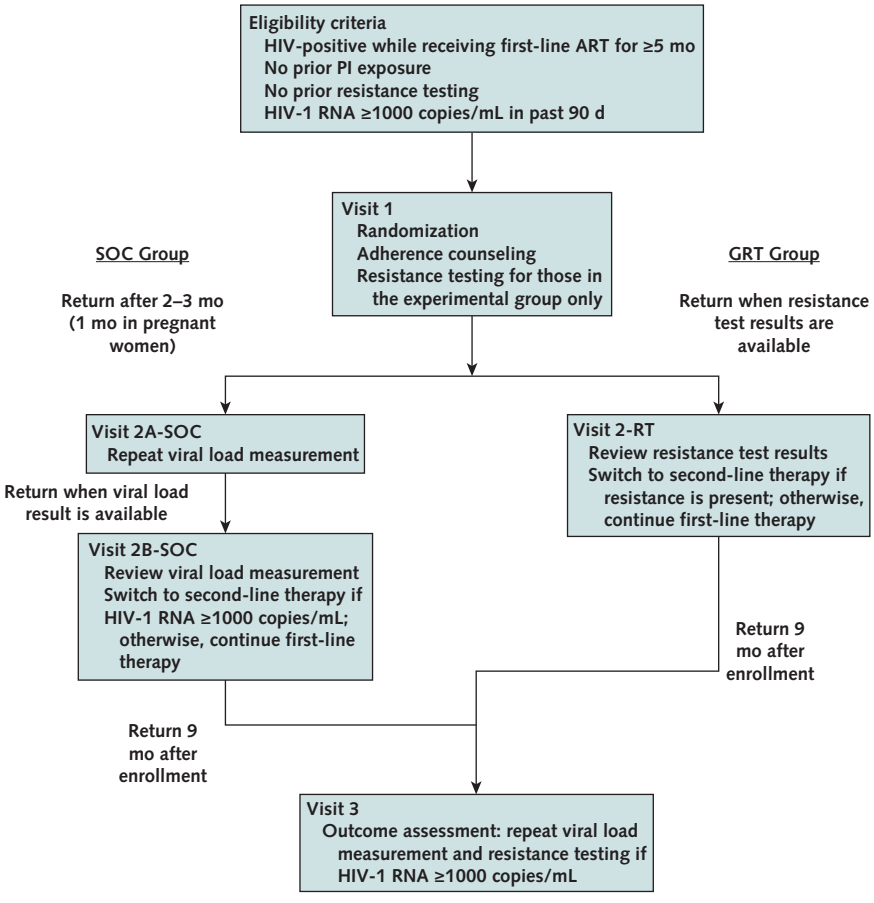
Author Contributions: Conception and design: M.J. Siedner, K. Ard, W. Muyindike, P. Moodley, T. Rautenberg, R.T. Gandhi, B. A. Johnson, H. Sunpath, M.B. Bwana, V.C. Marconi.
Analysis and interpretation of the data: M.J. Siedner, S. McCluskey, K. Ard, W. Muyindike, N. Musinguzi, M. Pillay, T. Rautenberg, R.T. Gandhi, B.A. Johnson, H. Sunpath, V.C. Marconi.
Drafting of the article: M.J. Siedner, J. Brijkumar, R.T. Gandhi, B. A. Johnson.
Critical revision of the article for important intellectual content: M.J. Siedner, M.Y.S. Moosa, S. McCluskey, K. Ard, P. Moodley, T. Rautenberg, G. George, H. Sunpath, V.C. Marconi.
Final approval of the article: M.J. Siedner, M.Y.S. Moosa, S. McCluskey, R.F. Gilbert, S. Pillay, I. Aturinda, K. Ard, W. Muyindike, N. Musinguzi, G. Masette, M. Pillay, P. Moodley, J. Brijkumar, T. Rautenberg, G. George, R.T. Gandhi, B.A. Johnson, H. Sunpath, M.B. Bwana, V.C. Marconi.
Provision of study materials or patients: M.J. Siedner, M.Y.S. Moosa, I. Aturinda, W. Muyindike, J. Brijkumar, H. Sunpath.
Statistical expertise: N. Musinguzi, B.A. Johnson.
Obtaining of funding: M.J. Siedner, T. Rautenberg, B.A. Johnson, V.C. Marconi.
Administrative, technical, or logistic support: M.J. Siedner, M.Y. S. Moosa, R.F. Gilbert, S. Pillay, I. Aturinda, K. Ard, G. Masette, M. Pillay, P. Moodley, H. Sunpath, V.C. Marconi.
Collection and assembly of data: M.J. Siedner, S. McCluskey, R. F. Gilbert, S. Pillay, I. Aturinda, W. Muyindike, N. Musinguzi, G. Masette, M. Pillay, P. Moodley, T. Rautenberg, H. Sunpath, M.B. Bwana.

Appendix Table. Logistic Regression Model of Predictors of Virologic Resuppression 9 Months After Study Enrollment*

Characteristic	Proportion Resuppressed, *n (%)*	Unadjusted Odds Ratio (95%CI)	Adjusted Odds Ratio (95% CI)
Sex			
Male	248/410 (60.5)	Reference	Reference
Female	271/430 (63.0)	0.99 (0.74–1.32)	0.94 (0.68–1.29)
Age at enrollment			
≤35 y	212/370 (57.3)	Reference	Reference
>35 y	307/470 (65.3)	1.52 (1.13–2.05)	1.57 (1.15–2.16)
Country			
Uganda	316/420 (75.2)	Reference	Reference
South Africa	203/420 (48.3)	0.31 (0.23–0.41)	0.27 (0.19–0.37)
CD4 count at enrollment			
≥0.200 × 10^9 cells/L	318/485 (65.6)	Reference	Reference
<0.200 × 10^9 cells/L	161/296 (54.4)	0.71 (0.52–0.98)	0.80 (0.57–1.11)
Viral load at enrollment			
≤10 000 copies/mL	272/408 (66.7)	Reference	Reference
>10 000 copies/mL	247/432 (57.2)	0.70 (0.91–2.25)	0.76 (0.55–1.05)
Self-reported treatment adherence at enrollment			
<100% in prior 30 d	362/592 (61.1)	Reference	Reference
100% in prior 30 d	157/248 (63.3)	1.39 (1.00–1.94)	1.36 (0.97–1.91)
Study group			
Standard of care	256/423 (60.5)	Reference	Reference
Genotypic resistance testing	263/417 (63.1)	1.11 (0.84–1.49)	1.18 (0.87–1.60)

* Results of mixed-effects logistic regression models with a random effect for randomization strata. Strata included study clinic, duration of antiretroviral therapy use (less than vs. more than 1 y), and pregnancy status at the time of enrollment.

Appendix Figure. Clinical trial flow diagram.



ART = antiretroviral therapy; GRT = genotypic resistance testing; PI = protease inhibitor; SOC = standard of care.