OPEN

High Prevalence of NRTI and NNRTI Drug Resistance Among ART-Experienced, Hospitalized Inpatients

Claire Bossard, MS,[a] Birgit Schramm, PhD,[a] Stephen Wanjala, MD,[b] Lakshmi Jain, MD,[c] Gisèle Mucinya, MD,[d] Valarie Opollo, PhD,[e] Lubbe Wiesner, MD,[f] Gilles van Cutsem, MD,[g,h] Elisabeth Poulet, MD,[a] Elisabeth Szumilin, MD,[i] Tom Ellman, MD,[g] and David Maman, MD[a]

Background: Patients hospitalized with advanced HIV have a high mortality risk. We assessed viremia and drug resistance among differentiated care services and explored whether expediting the switching of failing treatments may be justified.

Setting: Hospitals in the Democratic Republic of (DRC) Congo (HIV hospital) and Kenya (general hospital including HIV care).

Methods: Viral load (VL) testing and drug resistance (DR) genotyping were conducted for HIV inpatients ≥15 years, on first-line antiretroviral therapy (ART) for ≥6 months, and CD4 ≤350 cells/μL. Dual-class DR was defined as low-, intermediate-, or high-level DR to at least 1 nucleoside reverse transcriptase inhibitor and 1 non-nucleoside reverse transcriptase inhibitor. ART regimens were considered ineffective if dual-class DR was detected at viral failure (VL ≥1000 copies/mL).

Results: Among 305 inpatients, 36.7% (Kenya) and 71.2% (DRC) had VL ≥1000 copies/mL, of which 72.9% and 73.7% had dual-class DR. Among viral failures on tenofovir disoproxil fumarate (TDF)-based regimens, 56.1% had TDF-DR and 29.8% zidovudine (AZT)-DR; on AZT regimens, 71.4% had AZT-DR and 61.9% TDF-DR, respectively. Treatment interruptions (≥48 hours during past 6 months) were reported by 41.7% (Kenya) and 56.7% (DRC). Approximately 56.2% (Kenya) and 47.4% (DRC) on TDF regimens had tenofovir diphosphate concentrations <1250 fmol/punch (sub-optimal adherence). Among viral failures with CD4 <100 cells/μL, 76.0% (Kenya) and 84.6% (DRC) were on ineffective regimens.

Conclusions: Many hospitalized, ART-experienced patients with advanced HIV were on an ineffective first-line regimen. Addressing ART failure promptly should be integrated into advanced disease care packages for this group. Switching to effective second-line medications should be considered after a single high VL on non-nucleoside reverse transcriptase inhibitor–based first-line if CD4 ≤350 cells/μL or, when VL is unavailable, among patients with CD4 ≤100 cells/μL.

Key Words: HIV drug resistance, hospitalized patients, advanced HIV

(*J Acquir Immune Defic Syndr* 2021;87:883–888)

From the [a]Epicentre, Médecins Sans Frontières, Paris, France; [b]OCP, Médecins Sans Frontières, Nairobi, Kenya; [c]OCP, Médecins Sans Frontières, Homa Bay, Kenya; [d]OCB, Médecins Sans Frontières, Kinshasa, DRC; [e]Kenya Medical Research Institute/Center for Global Health Research, Kisumu, Kenya; [f]Division of Clinical Pharmacology, Department of Medicine, University of Cape Town, Cape Town, South Africa; [g]Southern African Medical Unit, Médecins Sans Frontières, Cape Town, South Africa; [h]Center for Infectious Disease Epidemiology and Research, University of Cape Town, South Africa; and [i]OCP, Médecins Sans Frontières, Paris, France.

This study was entirely funded by MSF France (Kenya) and MSF Belgium (DRC). The University of Cape Town Clinical PK Laboratory is supported in part by the Adult Clinical Trial Group (ACTG), by the National Institute of Allergy and Infectious Diseases (NIAID) of the National Institutes of Health under award numbers UM1 AI068634, UM1 AI068636, and UM1 AI106701; as well as the Infant Maternal Pediatric Adolescent AIDS Clinical Trials Group (IMPAACT), funding provided by National Institute of Allergy and Infectious Diseases (U01 AI068632), The Eunice Kennedy Shriver National Institute of Child Health and Human Development, and National Institute of Mental Health grant AI068632.

Meetings or conferences: CROI; March 7, 2019; Seattle, WA, MSF-Epicentre Scientific Days; September 2019; Paris, WHO (viral load guideline preparatory meetings), March 2020.

The authors have no conflicts of interest to disclose.

C.B., S.W., D.M., E.S., G.V.C., T.E., and E.P. conceived and designed the research study, C.B. and D.M. performed the research, and B.S., G.O., S.W., and L.J. contributed to implementation, V.O. and L.W. supervised the laboratory tests, respectively, in Kenya Medical Research Institute/Center for Global Health Research) (KMRI/CGHR) and University of Cape Town (UCT), C.B., and B.S. analyzed the data and wrote the first draft of the manuscript, and B.S., S.W., D.M. E.S., GVC, T.E., and E.P. contributed to result interpretation. All authors contributed to subsequent drafts and reviewed and approved the final manuscript.

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.jaids.com).

Submitted as a concise communication.

Correspondence to: Claire Bossard, MS, Doctors Without Borders, Deneb House, 368 Main Road Observatory, Cape Town, South Africa 7925 (e-mail: claire.bossard@epicentre.msf.org).

INTRODUCTION

In sub-Saharan Africa, many people living with HIV (PLHIV) who have already been treated are still hospitalized with advanced disease. Up to 78% of HIV inpatients in a Congolese cohort were antiretroviral therapy (ART)-exposed,[1] and the majority (59% and 64%) of highly immunocompromised Zambian and Kenyan patients (CD4 <200 cells/mm^3) had already taken ART when studied,[1,2] nearly half for more than 6 months. Mortality and opportunistic infections are frequent in this group: 20%–30% die while hospitalized.[3] This risk increases as CD4 counts drop,[1,4] and many of these

patients have unidentified first-line ART resistance because of a lack of access to viral load (VL) and genotype resistance testing.[5,6] Current World Health Organization[7] (WHO) guidelines advise that 2 consecutive VL readings above 1000 copies/mL (at least 3 months apart), with adherence counseling in-between, should be completed before switching a patient to second-line regimen. In practice, this can be a lengthy process with high rates of loss to follow-up at each step.[8,9]

Delayed switching to second-line ART was shown to be associated with elevated mortality, particularly in advanced disease.[10] Switching a failing regimen early is effective at reducing mortality, especially in patients with low CD4 counts.[11] Yet, switching to a second-line regimen, particularly when protease inhibitors are used, is not always uniformly beneficial and may result in higher pill burden, potential side-effects, and poorer adherence. To assess the need for more rapid switching from first- to second-line regimens for seriously ill patients, we measured viremia and genotypic drug resistance (DR) in ART-experienced advanced HIV patients in 2 hospitals' inpatient departments in Kenya and Democratic Republic of Congo (DRC).

METHODS

Study Population and Setting

We conducted a cross-sectional study in the Homa Bay County Teaching and Referral Hospital's inpatient departments in Kenya and the Center Hospitalier de Kabinda (CHK) in Kinshasa, DRC. These distinct sites represent a high prevalence (26.0%), high ART coverage (63%) site in Kenya and a low prevalence (1.6%), low ART coverage (33%) setting in DRC.[12,13]

Hospitalized PLHIV ≥15 years of age, on first-line ART ≥6 months, with CD4 ≤350 cells/μL were recruited from October to December 2017 in DRC and from February to July 2018 in Kenya. A minimum sample size of 216 was calculated based on an assumed 50% antiretroviral (ARV) resistance prevalence (expected outcome), assuming a non-response and laboratory examination failure rate of 10%. In Kenya, after 2.5 months of participant recruitment, fewer hospitalized patients with a high VL (≥1000 copies/mL) were found than expected. After exclusions for sampling and laboratory errors, the sample size was increased to 187 for that site. Ethical approval was granted by the Kenya Medical Research Institute Scientific and Ethics Review Committee in Kenya (including amendment), the Comité d'Ethique à la Recherche Scientifique in DRC, and Médecins Sans Frontières (MSF's) organizational Ethics Review Board (Protocol ID: 1743).

Data Collection

All admitted patients were screened for eligibility with Determine Rapid HIV-1/2 Antibody testing and, if positive, by Uni-Gold Rapid HIV testing (Trinity Biotech PLC). CD4 counts were determined by PIMA point-of-care testing (Alere, Germany). Standardized questionnaires (demographics, previous hospitalizations, and treatment history) were completed during hospitalization. Treatment interruption was defined as at least 1 self-reported treatment interruption of ≥48 hours in the past 6 months. Blood was taken for VL, resistance genotyping, and tenofovir diphosphate (TFV-DP) dried blood spot testing. Plasma VL testing was performed at CHK laboratory in DRC (Abbott m2000; HIV-1 RNA quantification range: 40–10,000,000 copies/mL), and at the Kenya Medical Research Institute/Center for Disease Control and Prevention HIV laboratory in Kisumu, Kenya (Cobas Ampliprep/Cobas Taqman HIV-1 test v.2.0, RNA quantification range: 20–10,000,000 copies/mL), where genotyping was performed on dried blood spot specimens with HIV-RNA ≥1000 copies/mL.[14] Intracellular TFV-DP concentration was assessed for participants on tenofovir disoproxil fumarate (TDF)-based regimens at the University of Cape Town using a modified version of Bushman et al.'s method.[15,16] In Kenya, this assessment was performed only for the group of patients included before the sample size was increased. Suboptimal treatment adherence was defined as intracellular TFV-DP concentration <1250 fmol per punch, corresponding to an optimal adherence level of 7 doses per week.[17,18]

DR was predicted using the Stanford HIV database algorithm, v8.8,[19] and DR was defined as any low-, intermediate-, or high-level resistance. Dual-class DR was defined as at least 1 DR in each drug class [nucleoside reverse transcriptase inhibitor (NRTI) and non-nucleoside reverse transcriptase inhibitor (NNRTI)]. A regimen-specific genotypic sensitivity score (sGSS) was calculated by assigning scores to each ARV within the respective regimen, using the 5 Stanford algorithm levels (between 0 for high-level resistance and 1 for fully susceptible ARV—maximum score 3).[20] ART regimens were considered ineffective if dual-class DR was detected at viral failure (1 high VL ≥1000 copies/mL).

Statistical Analysis

We calculated proportions with 95% confdence intervals (CIs) separately for the 2 sites except for DR mutations, which were combined. Combined univariate and multivariate logistic models explored predictors of dual-class DR after 1 high VL (sex, age, CD4 count, treatment duration, and treatment interruption of ≥48 hours in the past 6 months). Participants with no genotypic resistance results (n = 12) or missing treatment interruption data (n = 3) were excluded from this specific analysis. Stata v13 was used for analysis.[21]

RESULTS

In the 2 sites, 317 (24.1%) individuals met the eligibility criteria. Their demographic and clinical characteristics are detailed in Table 1. About half the participants (56.2% Kenya and 47.4% DRC) on a TDF-containing regimen had been suboptimally adherent (TFV-DP <1250 fmol/punch); 9.0% and 7.4% had a TFV-DP concentration below the limit of quantification. Treatment interruptions (at least once for ≥48 hours in the past 6 months) were reported by 41.7% (Kenya) and 56.7% (DRC), with the most recent lasting over 1 month for half of them (49.1% Kenya and 55.4% DRC).

Among all patients included, 36.7% in Kenya (69/187) and 71.2% in DRC (84/118) had a VL ≥1000 copies/mL.

TABLE 1. Demographic and Clinical Characteristics of Hospitalized Advanced HIV Inpatients in Kenya and the Democratic Republic of Congo, 2017–2018

	Kenya (N = 187)	DRC (N = 118)
Demographics and HIV care		
Female, n (%)	100 (53.5)	82 (69.5)
Age, yr, median (IQR)	37 (30–46)	40 (32–48)
Time on ART, yr, median (IQR)	4.0 (1.8–8.9)	5.3 (2.5–10.3)
Previous hospitalization in the past 3 months (2 missing in Kenya)	47 (25.1)	27 (22.9)
ART regimen, n (%)		
TDF/3TC/EFV	132 (70.6)	93 (78.8)
TDF/3TC/NVP	10 (5.3)	4 (3.4)
TDF/3TC/DTG	1 (0.5)	0
AZT/3TC/NVP	21 (11.2)	6 (5.1)
AZT/3TC/EFV	18 (9.6)	5 (4.2)
ABC/3TC/EFV	3 (1.6)	8 (6.8)
ABC/3TC/NVP	2 (1.1)	2 (1.7)
Treatment interruption, n/N (%)		
≥1 self-reported treatment interruption* previous 6 months (5 missing in DRC)	78/187 (41.7)	64/113 (56.7)
Adherence, n/N (%)		
TFV-DP concentration† <1250 fmol	50/89 (56.2)	45/95 (47.4)
TFV-DP concentration <700 fmol	26/89 (29.2)	33/95 (34.8)
CD4, cells/μL, n (%)		
Median (IQR)	135 (46–255)	69 (29–134)
200–350	67 (35.8)	19 (16.1)
100–199	45 (24.1)	28 (23.7)
51–99	26 (13.9)	18 (15.3)
≤50	49 (26.2)	53 (44.9)
VL ≥1000 copies/mL, n; % (CI)		
CD4 200–350 cells/μL	5; 7.5 (3.1 to 16.8)	8; 42.1 (22.4 to 64.7)
CD4 100–199 cells/μL	13; 28.9 (17.5 to 43.8)	18; 64.3 (45.1 to 79.8)
CD4 51–99 cells/μL	10; 38.5 (22.0 to 58.1)	11; 61.1 (37.5 to 80.4)
CD4 ≤50 cells/μL	41; 83.7 (70.5 to 91.7)	47; 88.7 (76.8 to 94.9)
Dual-class drug resistance, n/N; % (CI)		
Among VL ≥1000 copies/mL and		
CD4 200–350 cells/μL	2/3; 66.7 (31.1 to 99.7)	2/7; 28.6 (4.2 to 78.5)
CD4 100–199 cells/μL	8/12; 66.7 (32.9 to 89.1)	10/17; 58.8 (32.7 to 80.8)
CD4 51–99 cells/μL	8/10; 80.0 (37.8 to 96.3)	8/9; 88.9 (37.4 to 99.1)
CD4 ≤50 cells/μL	30/40; 75.0 (58.7 to 86.4)	36/43; 83.7 (68.9 to 92.3)

*Self-reported treatment interruption is defined as a disruption of for ≥48 h.
†TFV-DP concentrations <1250 fmol is considered suboptimal adherence (1250 fmol corresponds to seven doses per week).

HIV genotyping was successful for 141 patients (92.3%) with a high VL (65/69 in Kenya and 76/84 in DRC). HIV subtype A was most common in Kenya and DRC (n = 54, 78.9%; n = 21, 26.9%). In DRC, subtypes G (n = 13, 17.5%) and C (n = 7, 9.5%) were also found. DR mutations were detected in most genotyped patients with 1 high VL: At least 1 NRTI mutation was detected in 74.5% (both sites combined), 70.9% of all genotyped patients had 3TC resistance, 56.7% had TDF resistance, and 36.9% had zidovudine (AZT)-DR (Fig. 1A). DR to at least 1 NNRTI was detected in 81.5% (Kenya) and 89.5% (DRC) of genotyped patients. Overall, 85.8% also had cross-resistance to efavirenz (EFV). Dual-class resistance was present in 72.9% and 73.7% of Kenyan and Congolese patients, respectively. In Kenya and DRC, 73.8% and 75.4% had an sGSS <2 (maximum score of 3 if all 3 ARVs susceptible per genotypic resistance test). Approximately 26.2% and 6.9% had an sGSS of zero (all ARVs had high-level resistance). The median sGSS among genotyped patients was 0.5 (0.25–2.0).

For TDF-based regimens, more than half of those with genotyping results had TDF-DR (56.1%; n = 64) and about one-third had AZT-DR (29.8%; n = 34). For AZT-based regimens, the majority had AZT-DR (71.4%; n = 15) and TDF-DR (61.9%; n = 13). Overall, nearly one-third (29.1%; n = 41) of those with DR results had TDF + AZT dual-class DR.

The most common NRTI mutation was M184V (65.2%). The main thymidine analog mutations (TAMs) were T215F/Y (25.5%), M41L (24.1%), and D67N (14.9%);

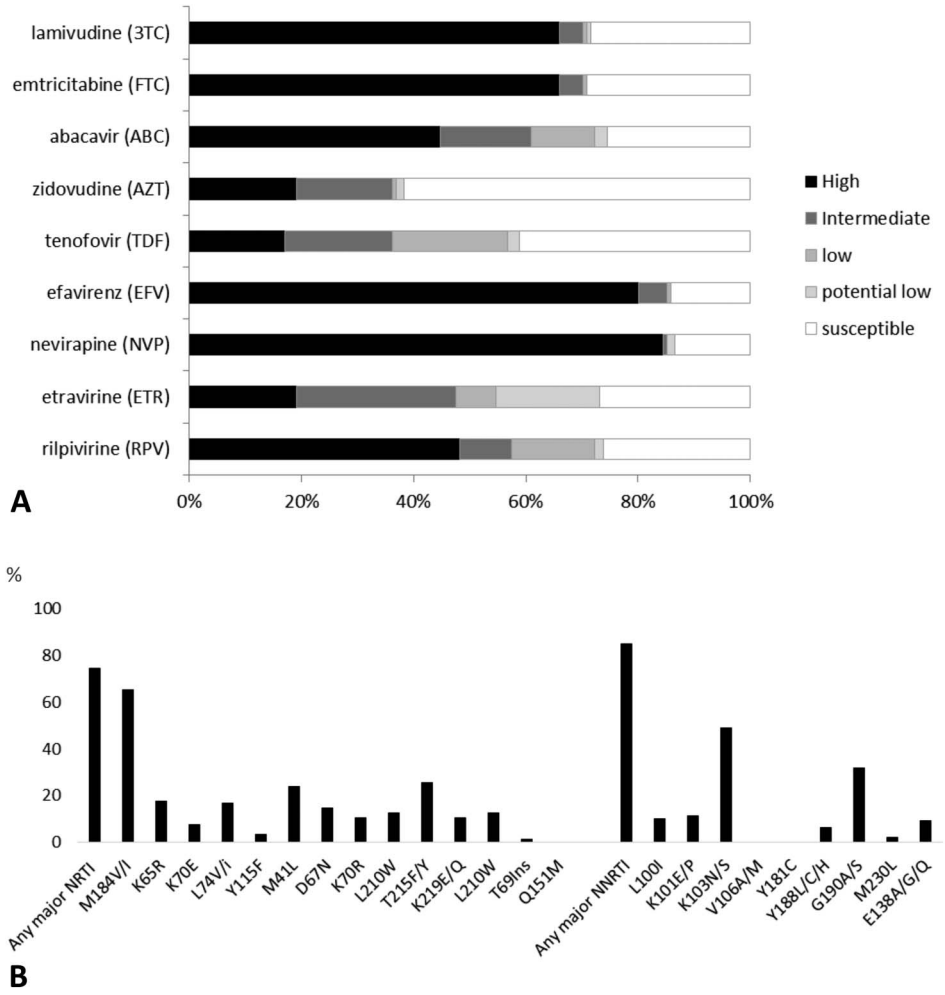

FIGURE 1. A, Predicted HIV resistance to nucleoside and non-nucleoside reverse transcriptase inhibitors in patients with VL ≥1000 copies/mL (n = 141) combined for both sites; (B) drug resistance mutations combined for both sites. Genotypic drug resistance was predicted using the Stanford HIV database algorithm (version 8.8).

19.1% had 3 TAMs or more. K65R was present in 17.7% and L74V/I in 17.0%. Within the NNRTI drug class, K103N/S was present in 48.9%, G190A/S in 31.9%, and K101E/P in 11.3% (Fig. 1B).

The prevalence of dual-class resistance was calculated by immunological and virological status (Table 1). Those with CD4 ≤50 cells/μL and CD4 51–<99 cells/μL had the highest prevalence of dual-class DR (75.0% and 83.7% in Kenya, 80.0% and 88.9% in DRC, respectively). When extrapolated to all severely immunocompromised patients (CD4 ≤50 cells/μL), 61.2% in Kenya and 67.9% in DRC were on an ineffective regimen regardless of their VL. In multivariate analysis, low CD4 count and treatment interruption(s) in the previous 6 months were predictors of dual-class DR (see Table, Supplemental Digital Content 1, http://links.lww.com/QAI/B649). The risk did not vary by treatment duration.

DISCUSSION

These results indicate high rates of suboptimal adherence, viral failure, and drug resistance in ART-experienced patients hospitalized with advanced HIV disease. They highlight the need to rapidly identify and promptly switch these patients to effective second-line medications.

A high proportion of these inpatients had a high VL (≥1000 copies/mL), and nearly all these viremic patients had dual-class DR. Among the study's severely immunocompromised participants (CD4 <100 cells/μL), nearly two-thirds were both viremic and had dual-class resistance. These patients have a particularly high mortality risk,[22,23] and the current 2-step WHO-recommended algorithm to diagnose treatment failure,[7] which delays switching to second-line ART by at least 3 months, seems unacceptably long. Switching regimens more rapidly could lead to immune restoration and decreased mortality, particularly in resource-poor settings where long delays are likely.[24] Poor adherence in this group (evidenced by participants' low intracellular TVF-DP concentrations and high self-reported treatment interruption rates) underscores the continued need for appropriate adherence counseling and psychosocial support, both during hospitalization and after discharge. Over a quarter of participants reported a previous hospitalization in the past 3 months (25.1% Kenya and 22.9% DRC), suggesting there were missed opportunities at inpatient level to address treatment failure. A qualitative study conducted simultaneously in both settings revealed that before

hospitalization, many patients had attempted to seek care at primary health facilities multiple times but remained unwell eventually self-presenting at hospital when their health deteriorated severely.[25]

Similar failure characteristics were seen in a recent Malawian study where HIV-DR was linked to an increased risk of postdischarge mortality.[26] Up to then, no specific data on the prevalence of high VL and drug resistance were available in this subgroup of hospitalized ART-experienced patients. Our results corroborate this evidence and further emphasize that virological failure and DR are significant concerns for this group. Moving forward, these factors should be considered at admission and addressed as soon as possible. The risk of IRIS on regimen switch with low CD4 and the need to monitor and support postswitch adherence should be well considered.

Slightly different inpatient characteristics in the 2 sites meant a higher proportion of severely immunocompromised (CD4 $<$100 cells/μL) patients were seen in DRC, where patients with HIV-related illnesses are admitted, than in Kenya, where we recruited from a general inpatient ward. Yet, regardless of site, prevalence of viral failure and low CD4 counts was high and associated with dual-class DR. Point-of-care VL and CD4 testing in hospitals is still not widely implemented; yet, these results emphasize their utility in low-resource, inpatient settings for effective patient management.[5,27,28]

Finally, the presence of inactive NRTI "backbone" medications in first-line ART regimens is concerning. Dual-drug resistance is common when NNRTI-based first-line regimens fail. Many countries, including Kenya and DRC, are now shifting to dolutegravir (DTG)-based regimens per WHO recommendation [for NNRTI-based first-line failures: optimized NRTI backbone + DTG; for TDF-based first-line failures: AZT/3TC/DTG; for AZT failures: TDF/3TC/DTG (TLD)].[29,30] These recommendations assume that patients with one failed NRTI backbone drug will still be susceptible to the other. Yet, in our study, nearly one-third of patients on TDF-based regimens were AZT-resistant, and nearly half of those on AZT-based treatments were resistant to TDF. Although phenotypic responses may differ from genotypic findings, the logic of systematically switching NRTI backbone medications needs close monitoring. The high AZT toxicity in anemic patients (common in advanced HIV cases) further complicates the systematic switching of TDF to AZT.[31] DTG-based second-line regimens may in fact support adherence among high-risk patients because of the drug's lower pill burden and overall tolerability, but clinical data on DTG effectiveness in the context of a fully resistant NRTI backbone are awaited.[32]

The study is limited by its cross-sectional design, which prevents drug resistance from being linked with individual treatment histories; reasons for hospitalization, postdischarge clinical outcomes including mortality, were not captured. Genotypic assays could only be conducted at recommended thresholds (VL $\geq$1000 copies/mL) and could not report resistance occurring at lower VL.[14]

CONCLUSIONS

Where testing VL and CD4 is available, immunocompromised (CD4 $\leq$350) hospitalized inpatients taking NNRTI-based first-line HIV treatments should switch to an effective second-line regimen as soon as a high VL is detected. Where VL testing is not available or frequently delayed, all severely immunocompromised hospitalized inpatients (CD4 $\leq$100) and anyone who is critically ill should switch to a second-line treatment regimen as soon as possible. It is acknowledged that this approach may be overcautious—it may end up putting a small number of (nonresistant) patients on second-line treatments unnecessarily. However, not taking this approach may create far greater harm when long delays prevent resistant patients from switching to effective treatment. In conjunction, inpatient and postdischarge counseling services should be integrated into advanced disease care packages to address adherence barriers in a timely manner, when rapid treatment switching is needed, and when second-line regimens are still protease inhibitor–based (and switching will possibly create a higher pill burden and side-effects).

Guidance on rapidly identifying patients on ineffective regimens and quickly and seamlessly transitioning these to effective ART need to be part of advanced disease protocols. Global health and local health policy makers must both be involved in producing guidance. Investment in adherence support to identify barriers of adherence early, a continuum from hospital to primary care, and VL and CD4 point-of-care testing, must also continue.

ACKNOWLEDGMENTS

The authors are grateful to all study participants and their caregivers, doctors, and nurses and support staff at CHK, DRC, and Homa Bay District Hospital, Kenya. The authors also thank the study teams in both sites, especially Gloria Omollo, Victor Adienge, Patrick Ngimbi Nsuka, Irene Etewa Eale, and Charles Luporte. The authors thank all staff on MSF teams who helped with the preparation and implementation of the study. The authors thank Emilie Venables and Rose Burns who implemented the qualitative component of the study. Medical editing was provided for this manuscript by MSF-France (Janet Ousley).

REFERENCES

1. Ousley J, Niyibizi AA, Wanjala S, et al. High proportions of patients with advanced HIV are antiretroviral therapy experienced: hospitalization outcomes from 2 Sub-Saharan African sites. *Clin Infect Dis.* 2018; 66(suppl 2):S126–S132.
2. Haachambwa L, Kandiwo N, Zulu PM, et al. Care continuum and postdischarge outcomes among HIV-infected adults admitted to the hospital in Zambia. *Open Forum Infect Dis.* 2019;6:ofz336.
3. Médecins Sans Frontières SAMU. *Left Behind by the HIV Response [Internet]*. 2017. Available at: https://samumsf.org/sites/default/files/2017-11/MSF Kinshasa Report ENG WEB.pdf. Accessed March 26, 2018.
4. Bisson GP, Ramchandani R, Miyahara S, et al. Risk factors for early mortality on antiretroviral therapy in advanced HIV-infected adults. *AIDS.* 2017;31:2217–2225.
5. UNICEF. *Point of Care HIV Diagnostics: Bringing Faster Results for Early and More Effective Treatment*. New York, NY: UNICEF; 2018. Available at: https://www.childrenandaids.org/sites/default/files/2017-08/POC%20Bringing%20Faster%20Results.pdf. Accessed June 30, 2019.
6. Ayalew M, Kumilachew D, Belay A, et al. First-line antiretroviral treatment failure and associated factors in HIV patients at the University

of Gondar Teaching Hospital, Gondar, Northwest Ethiopia. *HIV AIDS (Auckl.)* 2016;8:141–146.
7. World Health Organization. *Consolidated Guidelines on the Use of Antiretroviral Drugs for Treating and Preventing HIV Infection. Recommendations for a Public Health Approach*. Geneva, Switzerland: World Health Organization; 2016.
8. 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;23:1397–1404.
9. Medecins Sans Frontieres. *Southern African Medical Unit. Making Viral Load Routine*. Geneva, Switzerland: Médecins Sans Frontières (MSF); 2016. Available at: https://www.msf.org/sites/msf.org/files/making_viral_load_routine_part_1_programmatic_strategies.pdf. Accessed November 20, 2019.
10. Petersen M, Tran L, Geng E, et al. Delayed switch of antiretroviral therapy after virologic failure associated with elevated mortality among HIV-infected adults in Africa. *AIDS*. 2014;28:2097–2107.
11. Bell-Gorrod H, Fox MP, Boulle A, et al. The impact of delayed switch to second-line antiretroviral therapy on mortality, depending on definition of failure time and CD4 count at failure. *Am J Epidemiol.* 2020;189:811–819.
12. National AIDS. *STI Control Programme. Kenya HIV County Profiles*. Nairobi, Kenya: National AIDS and STI Control Programme (NASCOP); 2016. Available at: https://nacc.or.ke/wp-content/uploads/2016/12/Kenya-HIV-County-Profiles-2016.pdf. Accessed April 2, 2018.
13. World Health Organization. *Democratic Republic of Congo: Estimated Antiretroviral Therapy Coverage Among People Living With HIV. Global Health Observatory Metadata Registry*. 2015. Available at: http://apps.who.int/gho/data/view.main.23300?lang=en. Accessed August 23, 2018.
14. Mackie NE, Phillips AN, Kaye S, et al. Antiretroviral drug resistance in HIV-1-infected patients with low-level viremia. *J Infect Dis.* 2010;201:1303–1307.
15. Bushman L, Kiser J, Rower J, et al. Determination of nucleoside analog mono-, di-, and tri-phosphates in cellular matrix by solid phase extraction and ultra-sensitive LC-MS/MS detection. *J Pharm Biomed Anal.* 2011;56:390–401.
16. Zheng J, Rower C, McAllister K, et al. Intracellular tenofovir-diphosphate and EmtricitabineTriphosphate. *J Pharm Biomed Anal.* 2016;122:16–20.
17. Anderson P, Albert Y, Castillo-Mancilla J, et al. Intracellular tenofovir-diphosphate and EmtricitabineTriphosphate in dried blood spots following directly observed therapy. *Antimicrob Agents Chemother*. 2018;62:1–13.
18. Castillo-Mancilla J, Haberer J. Adherence measurements in HIV: new advancements in pharmacologic methods and real-time monitoring. *Curr HIV AIDS Rep.* 2018;15:49–59.
19. Standford University. *Major HIV-1 Drug Resistance Mutations*. Standford, CA: Standford University; 2017:3–4.
20. Boyd MA, Moore CL, Molina JM, et al. Baseline HIV-1 resistance, virological outcomes, and emergent resistance in the SECOND-LINE trial: an exploratory analysis. *Lancet HIV*. 2015;2:42–51.
21. *Statacorp Stata Stat Softw Release 13*. College Station, TX: StataCorp LLC; 2013.
22. Hoffmann CJ, Schomaker M, Fox MP, et al. CD4 count slope and mortality in HIV-infected patients on antiretroviral therapy: multi-cohort analysis from South Africa. *J Acquir Immune Defic Syndr*. 2013;63:34–41.
23. May MT, Vehreschild JJ, Trickey A, et al. Mortality according to CD4 count at start of combination antiretroviral therapy among HIV-infected patients followed for up to 15 years after start of treatment: collaborative cohort study. *Clin Infect Dis.* 2016;62:1571–1577.
24. Shroufi A, Van Cutsem G, Cambiano V, et al. Simplifying switch to second-line antiretroviral therapy in sub Saharan Africa: predicted effect of using a single viral load to define efavirenz-based first-line failure. *AIDS*. 2019;33:1635–1644.
25. Burns R, Wringe A, Odhoch L, et al. *Applying Social Stress Theory to Understand HIV Care Engagement Among Hospitalised Patients With Advanced HIV Infection in Rural Kenya*. Amsterdam, the Netherlands: Association for the Social Sciences and Humanities; 2018.
26. 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;7:620–628.
27. Drain P, Dorward J, Violette L, et al. *Point-of-Care Viral Load Testing Improves HIV Viral Suppression and Retention in Care*. Seattle, Washington: CROI; 2019.
28. Dorward J, Drain PK, Garrett N. Point-of-care viral load testing and differentiated HIV care. *Lancet HIV*. 2018;5:e8–e9.
29. World Health Organization (WHO). *Updated Recommandations on First-Line and Second-Line Antiretroviral Regimens and Post Exposure Prophylaxis and Recommandations on Early Infant Diagnosis of HIV*. 2019. Available at: https://apps.who.int/iris/bitstream/handle/10665/277395/WHO-CDS-HIV-18.51-eng.pdf?ua=1. Accessed November 13, 2020.
30. World Health Organization (WHO). *Update of First and Second Line Antiretroviral Regimens*. Geneva, Switzerland: WHO; 2019.
31. Volberding PA, Levine AM, Dieterich D, et al. Anemia in HIV infection: clinical impact and evidence-based management strategies. *Clin Infect Dis.* 2004;38:1454–1463.
32. Inzaule SC, Hamers RL, Doherty M, et al. Curbing the rise of HIV drug resistance in low-income and middle-income countries: the role of dolutegravir-containing regimens. *Lancet Infect Dis*. 2019;19:e246–e252.