Virological Failure and Drug Resistance in Patients on Antiretroviral Therapy After Treatment Interruption in Lilongwe, Malawi

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Background. Since 2004, Malawi has rapidly scaled up access to antiretroviral therapy (ART) in the national program following a public health approach with limited laboratory monitoring. We examined virological outcomes in patients with treatment interruption at 2 clinics of the Lighthouse Trust, Lilongwe, Malawi.

Methods. We evaluated patients who resumed first-line ART after having at least 1 treatment interruption documented in the electronic data system in 2008–2009. Viral load (VL) was analyzed at least 2 months after resumption of ART. For VL ≥1000 copies/mL, drug-resistance genotype was characterized using the Stanford database.

Results. Between June and November 2009, we enrolled 133 patients (58.7% female) with a mean age of 38.4 years. Mean duration of ART prior to treatment interruption was 14.3 months. After a minimum of 2 months following ART resumption, VL was undetectable in 81 (60.9%) patients, was 400–1000 copies/mL in 12 (9.0%) patients, and was ≥1000 copies/mL in 40 (30.1%) patients. Genotyping and drug-resistance testing were successfully performed for 36 of 40 patients, all carrying human immunodeficiency virus type 1 subtype C. Relevant mutations affecting nonnucleoside reverse transcriptase inhibitors were found in 32 of 133 (24.1%) patients and combined with relevant nucleoside reverse transcriptase mutations in 27 of 133 (20.3%) patients.

Conclusions. Virological failure combined with drug resistance after resumption of first-line ART occurred in 24.1% of the patients with treatment interruption, requiring a switch to protease inhibitor–based second-line therapy. Patients with treatment interruption should receive VL assessment after resumption of ART to detect treatment failure and to reduce development and spread of drug resistance.

Following the introduction of free antiretroviral therapy (ART) in 2004, Malawi implemented a rapid ART scale-up based on a public health approach [1]. By the end of 2009, approximately 200 000 patients had been initiated on ART [2]. Effective ART for patients living with human immunodeficiency virus (HIV) is primarily determined by drug adherence [3]. Patients with low drug adherence are expected to be more likely to develop drug resistance [4–12]. Equally important for the success of ART programs is a high degree of retention in care [13]. A systematic review of sub–Saharan Africa reported overall retention averaging 70%–77% at 24 months after ART initiation [14]. For Malawi, Hedt et al showed that less than half of public ART sites reach the World Health Organization (WHO) target of 70% patient retention 12 months after ART initiation [15]. Attempts to improve both patients' retention in care and drug adherence are considered preventive in terms of treatment failure and development of drug resistance [16]. The development of strategies for improved drug adherence and optimized retention in care consequently needs to be complemented by tools to detect treatment failure [17–19]. In Malawi, the evaluation of ART failure is routinely

Received 24 November 2011; accepted 10 April 2012; electronically published 9 May 2012.

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Clinical Infectious Diseases 2012;55(3):441–8

DOI: 10.1093/cid/cis438

based on clinical or immunological criteria, if CD4 cell count is available. Although confirmatory viral load (VL), defined as HIV RNA level, testing is possible at central hospitals, resistance testing is only available in research settings through international collaborations. Therefore, data on virological failure and patterns of HIV drug-resistance mutations in Malawi's public health–based ART programs remain scarce [20, 21].

According to Malawi's national treatment guidelines [22], patients with treatment interruption resume their previous regime without regard for CD4 cell count trend or specified VL determination after resumption of therapy. In 2009, first-line ART consisted of stavudine (d4T), lamivudine (3TC), and nevirapine (NVP) (Triomune) as a fixed-drug combination. In case of toxicity, zidovudine (ZDV) could be substituted for d4T, or efavirenz (EFV) could be substituted for NVP. Less than 1% of patients receive second-line therapy consisting of ZDV, 3TC, tenofovir (TDF), and lopinavir/ritonavir (LPV/r) [2].

The aim of this study was to determine the prevalence of virological failure and patterns of drug resistance among patients with treatment interruption and to evaluate the current practice of resuming first-line ART.

PATIENTS AND METHODS

Study Setting

The study was conducted at the 2 ART clinics in Lilongwe, Malawi, of the Lighthouse Trust, 1 of the largest public ART providers in the country. Both ART sites apply national treatment guidelines and internal standard operation procedures and are referred to as "Lighthouse" in the following. Since 2004, Lighthouse has provided ART and regular clinical reviews free of charge in line with the national scale-up program and the framework of the WHO Universal Access strategy [18, 23, 24]. Lighthouse routinely uses a real-time electronic data system (EDS) for the collection of patient data and documentation of all visits, prescriptions, and dispensing. At each clinic visit, adherence is assessed via pill count. Adherence levels are calculated in the EDS, and patients with adherence <95% are referred to an adherence counseling session. In order to improve patient retention, the Back-to-Care (B2C) program for early active follow-up of patients was initiated in 2006. The program uses data from the EDS to identify patients who are ≥21 days overdue for their scheduled appointment, defined as patients lost-to-follow-up (LTFU). Patients LTFU are contacted via mobile phone and/or through home visits with motorcycles in order to determine their actual ART status. The status is recorded as either dead, unsuccessful tracing attempt, transfer to another ART facility, uninterrupted therapy with alternate ART source, never started ART, treatment gaps, or complete stop of all prescribed antiretroviral drugs. All patients found alive and not transferred to another ART facility were encouraged to return and continue therapy. The B2C program and factors associated with returning patients LTFU to the clinic were previously described by Tweya et al [25].

Study Population

This study focused on patients with treatment interruption between January 2008 and November 2009 who decided to return to the clinic for ART resumption. Treatment interruption was defined as ART status of "treatment gaps" or "stop of ART" documented through the B2C program in patients who missed their scheduled appointment for ≥21 days. In addition, drug history and treatment interruption were again confirmed by patient interview prior to study inclusion. Eligible patients were aged ≥15 years, resumed first-line therapy, and had no previous exposure to protease inhibitor–based regimes (Figure 1).

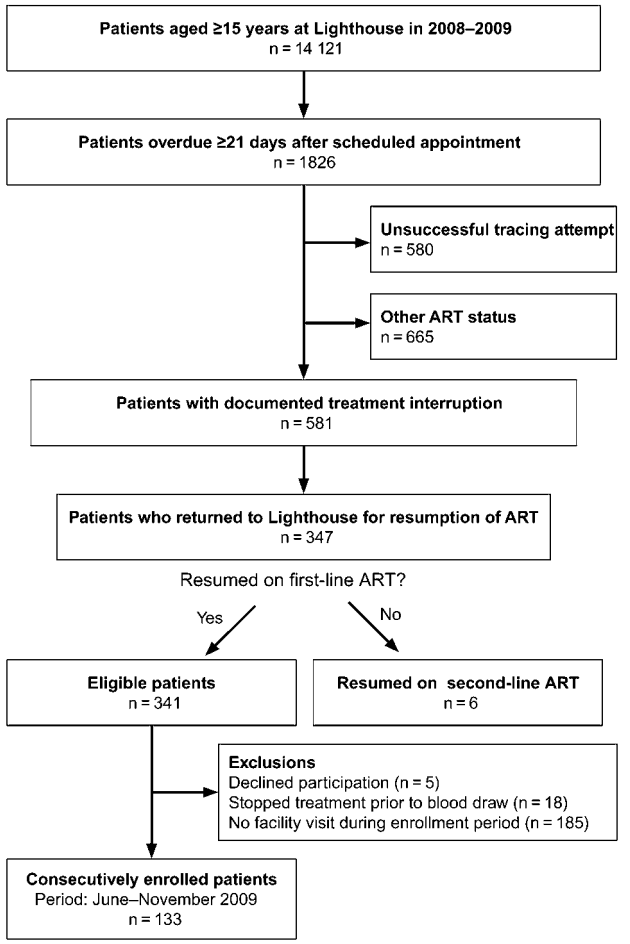

Figure 1. Study population and sample selection. Eligibility criteria were aged ≥15 years, treatment interruption of ≥21 days (between January 2008 and November 2009), resumption of first-line regime, and no previous exposure to protease inhibitors. Abbreviation: ART, antiretroviral therapy.

Laboratory Evaluations

CD4 cell count and VL were determined after a minimum of 2 months following resumption of ART. Virological failure was defined as VL ≥1000 copies/mL. In addition, samples with VL ≥1000 copies/mL were genotypcially tested for HIV drug resistance.

CD4 cell counts were measured at the Laboratory of the University of North Carolina (UNC) project in Lilongwe, Malawi, using flow cytometry by the EPICS-MCL Beckman Coulter Pan-Leuco Gating method. Plasma specimens were stored at −80°C at the UNC project until shipment to Germany for further laboratory evaluations.

Viral load was measured at the Kamuzu Central Hospital Laboratory, Lilongwe, Malawi, using Roche Amplicor HIV-1 RNA Monitor kit version 1.5 (Roche Molecular Diagnostics). For quality assurance, VL was additionally determined in every 10th study sample at the Department of Infectious Diseases (Virology) at the University of Heidelberg, Germany, using Abbott Real Time HIV-1 kit (automatic sample preparation; Abbott m2000sp) and real-time polymerase chain reaction (PCR) (Abbott m2000rt), with a detection limit of 40 copies/mL. Both laboratories use internal assay controls provided by the manufacturers (eg, negative low VL and high VL controls). Both laboratories regularly participate in national or international external quality control proficiency panels to assure high quality in VL determination.

The HIV genotype characterization was performed in patients with VL ≥1000 copies/mL at the University Hospital Heidelberg, Germany, Department of Infectious Diseases (Virology). RNA extraction and reverse-transcriptase PCR were performed using the Viroseq kit (Abbott Diagnostics) according to a standard protocol. Sequences were submitted to the Stanford University HIV Drug Resistance Database [26]. This tool compares HIV sequences to subtype B isolates shown to confer resistance to anti-HIV drugs. The Stanford Database classifies identified mutations translating into susceptible, potential low-, low-, intermediate-, and high-level resistance. In this study, resistance was defined as the presence of at least 1 major resistance-related mutation.

Data Collection and Statistical Analysis

Baseline characteristics of patients, including WHO stage at ART initiation, duration of ART prior to interruption, ART regimen, and laboratory data, were extracted from the EDS and managed in a Microsoft Access database. The B2C data and ART status were recorded on a standardized questionnaire and double entered into a Microsoft Access database. Statistical analysis was performed using STATA11. Simple descriptive statistics included means, medians, and proportions, as appropriate. Student t test and χ^2 test were applied as required. We compared the enrolled patients with the overall group of eligible patients. Univariate logistic regression with virological failure as the dependent variable was performed to evaluate factors associated with virological failure.

Ethical Approval

Informed consent was obtained from patients prior to recruitment for the study. Because the threshold age for independent consent for ART is 15 years according to the Malawian guidelines, we included patients aged 15–18 years. The study was approved by the Malawi National Health Science Research Committee and the Ethical Research Board of the University Clinic of Heidelberg, Germany.

RESULTS

Study Population and Baseline Characteristics

Between 2008 and 2009, 14 121 patients aged ≥15 years received antiretroviral drugs at Lighthouse. Within this period, 1826 (12.9%) patients were identified as overdue for their scheduled appointment by ≥21 days. Early active follow-up through the B2C program ascertained the actual ART status of the patients LTFU (Figure 1) and identified those patients with treatment interruption (n = 581). Only a subset of the total number of patients with treatment interruption (n = 347) returned to the clinic for continuation of therapy. Due to logistical time and financial constraints, recruitment was restricted to eligible patients consulting the clinic between June and November 2009. In total, 133 patients, equaling 39.0% of patients fulfilling the eligibility criteria, were included. The recruited patients did not differ significantly from the total number of patients fulfilling eligibility criteria with regard to basic demographics (gender, age, and occupation), WHO stage at ART initiation, ART regime, duration of therapy prior to interruption, and period of interruption (ie, time between missed appointment date and resumption of ART) (Table 1).

The drug history of study patients (n = 133) revealed that 91% of patients interrupted ART once, 7.5% interrupted ART twice, and 1.5% interrupted ART 3 times between 2008 and 2009. Treatment interruption was characterized as a complete stop of ART in 90.2% and as treatment gaps in 9.8% of the patients. All study patients resumed their previous first-line therapy, for which 25 patients had ZDV substituted for d4T, and 6 patients had EFV substituted for NVP due to toxicity (Table 1).

Virological Failure

Following resumption of ART, VL was determined after a minimum of 2 months (range 2–19; median 6.8). Viral suppression (≤400 copies/mL) was found in 81 (60.9%) patient; 12 (9.0%) patients had a VL of 400–1000 copies/mL, and 40

Table 1. Total Cohort of Eligible Patients With Treatment Interruption Resuming Therapy Between 2008 and 2009 (n = 341) Versus Study Sample (n = 133)

Characteristic	Total Cohort (n = 341)	Study Sample (n = 133)
Female	199 (58.4)	78 (58.7)
Age, median years (IQR)	34 (27–41)	33 (27–40)
WHO clinical stage at initiation of ART		
1 or 2	44 (12.9)	19 (14.3)
3	183 (53.7)	66 (49.6)
4	65 (19.1)	25 (18.8)
Unknown	49 (15.0)	23 (17.3)
ART regime at time of resumption of therapy		
D4T/3TC/NVP	263 (77.1)	102 (76.7)
ZDV/3TC/NVP	51 (15.0)	25 (18.8)
D4T/3TC/EFV	27 (7.9)	6 (4.5)
Duration of ART,[a] median months (IQR)	10 (4–19)	9 (4–18)
Period of interruption,[b] median days (IQR)	61 (43–87)	61 (44–88)

Data are No. (%) of patients, unless otherwise specified.

Abbreviations: 3TC, lamivudine; ART, antiretroviral therapy; D4T, stadivudine; EFV, efavirenz; IQR, interquartile range; NVP, nevirapine; WHO, World Health Organization; ZDV, zidovudine.

[a] Duration of ART prior to treatment interruption.

[b] Period of interruption: time between missed appointment date and resumption of ART.

Table 2. Characteristics of Patients With Virological Failure

Characteristic	Virological Failure (n = 40)
Females	25 (62.5)
Age, median years (IQR)	29 (24–38)
<25	12 (30.0)
≥25	28 (70.0)
CD4 cell count,[a] median cells/μL (IQR)	241 (168–349)
<50	1 (2.8)
51–250	18 (50.0)
251–350	9 (25.0)
>350	8 (22.2)
HIV RNA, median copies/mL (IQR)	79 542 (3664–98 636)
<10 000	16 (40.0)
10 001–100 000	15 (37.5)
>100 000	9 (22.5)
Duration of ART,[b] median months (IQR)	9 (4–15)
0–12	27 (67.5)
>12–24	6 (15.0)
>24–36	2 (5.0)
>36	5 (12.5)
Period of interruption,[c] median days (IQR)	59 (46–87)
Duration of ART postresumption, median months (IQR)	4 (2–8)

Virological failure is defined as human immunodeficiency virus type 1 (HIV-1) RNA ≥1000 copies/mL. Data are No. (%) of patients, unless otherwise specified.

Abbreviations: ART, antiretroviral therapy; IQR, interquartile range; RNA, ribonucleic acid.

[a] In four patients CD4 cell count failed.

[b] Duration of ART prior to treatment interruption.

[c] Period of interruption: time between missed appointment date and resumption of ART.

(30.1%) had a VL ≥1000 copies/mL. In patients with virological failure (n = 40; 62.5% female), the median CD4 cell count, VL, and time on ART were 241.5 cells/μL, 79 542 copies/mL, and 8.5 months, respectively (Table 2). The comparison of VL determination of patients' samples in the Malawian and German laboratories revealed a high degree of correspondence. Variables evaluated in the univariate logistic regression for association with virological failure included age, sex, WHO stage at ART initiation, duration of therapy prior to interruption, period of interruption, and number of interruptions in 2008–2009. However, none of these variables discriminated significantly between patients with or without virological failure.

Patterns of Drug Resistance

The HIV drug-resistance genotype characterization was performed on the 40 (30.1%) samples from patients who had VL ≥1000 copies/mL. Amplification failed in 4 samples, leaving 36 (27.1%) for analysis. All viral strains were HIV-1 subtype C. In 4 samples, no mutations were identified. In the remaining 32 (24.1%) samples, all had nonnucleoside reverse transcriptase inhibitor (NNRTI) mutations, and 27 (20.3%) had additional nucleoside reverse transcriptase inhibitor (NRTI) mutations. The median number of NNRTI mutations was 2 (range 1–4), with 13 samples having 1, 10 samples having 2, and 9 samples having 3 or 4 mutations (see Supplementary Table 1). The most frequent NNRTI mutations were Y181C (n = 16), K103N (n = 13), and G190A (n = 9). M184V or M184I mutations were present in the virus of 25 of 32 samples (78.1%), although never as the only mutation (Table 3). Combination of M184V and NNRTI mutation occurred in 46.9% (15 of 32) of samples with mutations. A thymidine analogue mutation (TAM)–containing virus was found in 21.9% (7 of 32) of samples with mutations, mostly combined with M184V and NNRTI mutations. The most frequent TAMs were L210W (n = 2) and T215Y (n = 2). Only 1 sample contained 2 TAMs. One sample showed a T69 insertion (see Supplementary Table 2). Of all patients with

Table 3. Mutations Associated With Drug Resistance in Patients With HIV RNA Levels ≥1000 copies/mL (n = 36[a])

Mutation	No.
Resistance mutation	32
NRTI	27 (84%)[b]
M184V/I	25
L210W	2
T215Y	2
T69ins	1
M41L	1
D67N	1
K65R	1
V75I	1
K70R	1
V118I	1
NNRTI	32 (100%)[b]
Y181C	16
K103N	13
G190A	9
H221Y	8
K101E	4
A98G	3
Y188C	2
V108I	2
K103S	1
V179D	1
Y181V	1
V106M	1
V106A	1
Combined NRTI/NNRTI mutations	27 (84%)
No resistance mutation	4

Bold normal font indicates major mutations. Bold italicized font indicates thymidine analogue mutations.

Abbreviations: HIV, human immunodeficiency virus; NNRTI, nonnucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor.

[a] In 4 patients, genotyping failed.

[b] Subjects with ≥1 mutation were counted only once.

drug-resistance mutations, only 3 patients had exposure to ZDV, of whom 1 patient showed TAMs, and no patient had exposure to EFV.

Drug resistance–related mutations among the patients with major NRTI (n = 27; 20.3%), NNRTI (n = 32; 24.1%) or both (n = 27; 20.3%) mutations led to resistance, particularly against NVP, EFV, and 3TC (Figure 2). In addition, more than half of the patients (19 of 32; 59.4%) were predicted to harbor drug resistance to etravirine, mainly based on single or combined Y181C (n = 16), G190A (n = 9), H221Y (n = 8), and/or K101E (n = 4) mutations.

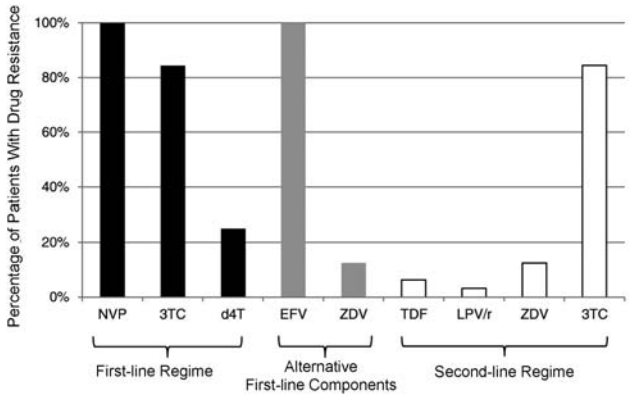

Figure 2. Patterns of mutation-related drug resistance (n = 32). This figure includes only patients with detectable resistance mutations (n = 32). Abbreviations: 3TC, lamivudine; D4T, stadivudine; EFV, efavirenz; LPV/r, lopinavir/ritonavir; NVP, nevirapine; TDF, tenofovir; ZDV, zidovudine.

DISCUSSION

In this study, we determined the virological outcome and drug resistance of a specific group of patients with treatment interruption followed by resumption of their previous first-line regime at Lighthouse, Lilongwe, Malawi. Although the majority of these patients had virological suppression, almost one-third (30.1%) had previously unnoticed virological failure, which was associated in 24.1% with drug resistance to first-line regime.

Regarding treatment guidelines including ART regimes and basic treatment monitoring, care at Lighthouse is representative for Malawi [27]. However, its capacity regarding the number of treated patients, quality management, and monitoring/evaluation exceeds the average ART center. Additional features like the early active follow-up (B2C) program aim to improve retention in care. Remarkably, only 4.1% of the overall treatment cohort interrupted ART. This compares favorably with other settings where figures of patients LTFU are higher [28]. In addition, most programs are not tracing patients LTFU to further differentiate their ART status as it is done by the B2C program. Therefore, the B2C program allows recognition and documentation of treatment interruption. Interestingly, in two-thirds of our patients, treatment interruption occurred within the first year of ART suggesting special support is necessary within this period [25].

Among the study patients resuming first-line therapy, 60.9% suppressed viral replication. Of the remaining patients with VL ≥1000 copies/mL and a genotyping result, almost all patients (32 of 36; 88.8%) had drug-resistance mutations, most of them (27 of 32; 84.4%) showing combined NRTI/NNRTI mutations. The pattern of drug resistance reflects the

expected spectrum when using the standard first-line ART of 3TC, d4T, and NVP and corresponds with similar results in the Malawian ART program 12 months posttreatment resistance survey (Malawi HIV-DR survey) [29] and data from sub–Saharan Africa [30]. The proportion of predicted resistance to etravirine (19 of 32; 59.4%) is higher than in other settings [31]. However, in our study group we did not see the emergence of K65R, K70E, Q151M mutations or extensive TAMS, as previously described for the same region in Malawi [32], possibly due to a shorter duration of first-line drug use. A study in Soweto, South Africa, evaluating risk factors for viremia and drug resistance among long-term recipients of ART in South Africa, found a different distribution for NNRTI mutations, also likely due to the longer duration of therapy and the predominance of EFV-containing regimens [33]. In our study, the identified drug-resistance patterns led to a loss of the currently available NNRTI options and limited NRTI treatment options, but left all patients the option for Malawi's current second-line treatment, consisting of ZDV, 3TC, TDF, and LPV/r.

This study highlights the risk of resistance development associated with treatment interruption [34, 35]. Patients with treatment interruption represent a high-risk group for virological failure and drug resistance and differ from the general treatment cohort surveyed through the Malawi HIV-DR survey [29]. The proportion of our patients harboring resistance mutations (24.1%) is almost 4-fold higher than the national mean of 6.1% and the site-specific mean at Lighthouse of 7.3% found in the Malawi HIV-DR survey [29]. None of our study patients were included in this survey. These large disparities suggest that the B2C program correctly identifies patients with higher risk of drug resistance compared with the overall cohort at Lighthouse.

A predicting factor for virological failure could not be established within the group of study patients. This may be due to the overall small number of patients with virological failure; it concurs with the findings in Soweto, South Africa, where also no association of viremia was seen with sex, age, and duration of ART [33].

The challenge to predict treatment failure using nonlaboratory algorithms underscores the importance of VL testing. Since the 2011 revision of Malawi's treatment guidelines, VL determination has been introduced for routine VL monitoring in predefined intervals, in case of suspected treatment failure, and prior to the switch to second-line ART [22].

We acknowledge the limitations of the study. For most of the patients not returning to care (nonreturners), the outcome with regard to resistance remains unknown. Although our study sample shared baseline characteristics with the total cohort of eligible patients, unmeasured factors associated with failure to return may limit the representativeness of the results. We assume that the rate of resistance development among nonreturners is at least as high as among patients resuming ART.

Among patients with VL of 400–1000 copies/mL, some may suppress if tested later, some could represent viral blips, and others may develop virological failure.

Our findings may result in practical implications for patient management and monitoring following treatment interruption for the B2C program and for the ART program at large. There is a need for qualified mechanisms to monitor VL after a minimum of 2–3 months following resumption of first-line ART. By then, insufficient reduction of VL (≥1000 copies/mL) would imply a high probability of treatment failure requiring a switch to the protease inhibitor–based second-line ART. With respect to the resource-constrained setting, additional testing for drug resistance at a program level may be unnecessary given the high association of drug resistance and virological failure, especially because the resulting regime change remains the same. Prerequisites for the implementation of VL monitoring include identification of patients interrupting ART and availability of VL testing and functioning reporting systems in public health ART programs [8–12].

Conclusions

We conclude from our data that viral suppression occurs after resumption of first-line ART, but a considerable percentage of patients develop HIV drug resistance. Thus, avoidance of treatment interruption is key and requires ART programs to provide custom-tailored solutions for the variety of patient needs (eg, adherence counseling) [36]. Emphasis must be put on the identification of those at risk for treatment interruption, especially during the first year of treatment. In our view, the findings justify the current practice of resuming first-line ART following treatment interruption in Malawi; however, this should be done only in conjunction with VL monitoring. Clear algorithms for VL monitoring need to be implemented, as suggested by the revised Malawian ART guidelines, in order to improve clinical outcomes and to avoid further spread of drug resistance.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online (http://cid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Acknowledgments. We are very appreciative of the support from the Ministry of Health (MoH), particularly Dr Andreas Jahn (Department of HIV/AIDS in MoH) for support in the development of the concept for the study and Dr Nellie Wadonda-Kabondo (Epidemiology Unit in MoH) for sharing prepublished data of the HIV Drug Resistance Surveillance Survey.

We would like to thank the staff of the Lighthouse clinic and the B2C team, the laboratory staff at UNC Project, particularly Debbie Kamwendo and Robert Krysiak, the laboratory staff at the Kamuzu Central Hospital, particularly Lloyd Nyangulu, and the laboratory staff at the University of Heidelberg, particularly Angelika Schillak, for supporting genotypic analyses.

Author contributions. F. N., H. T., J. L., P. S., and S. P. conceived and designed the study. J. L., H. T., F. N., J. M., S. P., and T. C. performed the study. F. N., H. T., H. R., J. L., M. C. H., P. S., and S. P. contributed to the analysis and writing of the paper. J. L. wrote the first draft. The first and last author contributed equally to the manuscript. All authors approved the final manuscript.

Financial support. This work was supported by a grant from the Hector Foundation, Mannheim, Germany. The funding agency had no influence on concept, conduction, analysis, and the decision to publish the study.

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Harries AD, Zachariah R, Jahn A, Schouten EJ, Kamoto K. Scaling up antiretroviral therapy in Malawi-implications for managing other chronic diseases in resource-limited countries. J Acquir Immune Defic Syndr **2009**; 52(Suppl 1):S14–6.
2. Ministry of Health Malawi. ART report 2009 Q4. Available at: http://www.hivunitmohmw.org/Main/AntiretroviralTherapy. Accessed 2 November 2011.
3. Bärnighausen T, Chaiyachati K, Chimbindi N, Peoples A, Haberer J, Newell ML. Interventions to increase antiretroviral adherence in sub–Saharan Africa: a systematic review of evaluation studies. Lancet Infect Dis **2011**; 11:942–51.
4. Glass TR, De GS, Weber R, et al. Correlates of self-reported non-adherence to antiretroviral therapy in HIV-infected patients: the Swiss HIV Cohort Study. J Acquir Immune Defic Syndr **2006**; 41: 385–92.
5. Antinori A, Cozzi-Lepri A, Ammassari A, et al. Relative prognostic value of self-reported adherence and plasma NNRTI/PI concentrations to predict virological rebound in patients initially responding to HAART. Antivir Ther **2004**; 9:291–6.
6. Carrieri MP, Raffi F, Lewden C, et al. Impact of early versus late adherence to highly active antiretroviral therapy on immuno-virological response: a 3-year follow-up study. Antivir Ther **2003**; 8:585–94.
7. Garcia de OP, Knobel H, Carmona A, Guelar A, Lopez-Colomes JL, Cayla JA. Impact of adherence and highly active antiretroviral therapy on survival in HIV-infected patients. J Acquir Immune Defic Syndr **2002**; 30:105–10.
8. Keiser O, Anastos K, Schechter M, et al. Antiretroviral therapy in resource-limited settings 1996 to 2006: patient characteristics, treatment regimens and monitoring in sub–Saharan Africa, Asia and Latin America. Trop Med Int Health **2008**; 13:870–9.
9. Kiguba R, Byakika-Tusiime J, Karamagi C, Ssali F, Mugyenyi P, Katabira E. Discontinuation and modification of highly active antiretroviral therapy in HIV-infected Ugandans: prevalence and associated factors. J Acquir Immune Defic Syndr **2007**; 45:218–23.
10. Nachega JB, Hislop M, Dowdy DW, Chaisson RE, Regensberg L, Maartens G. Adherence to nonnucleoside reverse transcriptase inhibitor-based HIV therapy and virologic outcomes. Ann Intern Med **2007**; 146:564–73.
11. Parienti JJ, Massari V, Descamps D, et al. Predictors of virologic failure and resistance in HIV-infected patients treated with nevirapine- or efavirenz-based antiretroviral therapy. Clin Infect Dis **2004**; 38:1311–6.
12. Ramadhani HO, Thielman NM, Landman KZ, et al. Predictors of incomplete adherence, virologic failure, and antiviral drug resistance among HIV-infected adults receiving antiretroviral therapy in Tanzania. Clin Infect Dis **2007**; 45:1492–8.
13. USAID Aidstar-One project. Patel A, Hirschhorn L, Fullem A, Ojikutu B, Oser R. Adult adherence to treatment and retention in care. Available at: http://www.aidstar-one.com/sites/default/files/AdultAdherenceTB_Final.pdf. Accessed: 4 April 2012.
14. Fox MP, Rosen S. Patient retention in antiretroviral therapy programs up to three years on treatment in sub–Saharan Africa, 2007–2009: systematic review. Trop Med Int Health **2010**; 15(Suppl 1):1–15.
15. Hedt BL, Wadonda-Kabondo N, Makombe S, et al. Early warning indicators for HIV drug resistance in Malawi. Antivir Ther **2008**; 13 (Suppl 2):69–75.
16. Rosen S, Fox MP, Gill CJ. Patient retention in antiretroviral therapy programs in sub–Saharan Africa: a systematic review. PLoS Med **2007**; 4:e298.
17. Calmy A, Ford N, Hirschel B, et al. HIV viral load monitoring in resource-limited regions: optional or necessary? Clin Infect Dis **2007**; 44:128–34.
18. Gilks CF, Crowley S, Ekpini R, et al. The WHO public-health approach to antiretroviral treatment against HIV in resource-limited settings. Lancet **2006**; 368:505–10.
19. Sungkanuparph S, Manosuthi W, Kiertiburanakul S, Piyavong B, Chumpathat N, Chantratita W. Options for a second-line antiretroviral regimen for HIV type 1-infected patients whose initial regimen of a fixed-dose combination of stavudine, lamivudine, and nevirapine fails. Clin Infect Dis **2007**; 44:447–52.
20. Kamoto K, Aberle-Grasse J. Surveillance of transmitted HIV drug resistance with the World Health Organization threshold survey method in Lilongwe, Malawi. Antivir Ther **2008**; 13(Suppl 2):83–7.
21. Maphalala G, Okello V, Mndzebele S, et al. Surveillance of transmitted HIV drug resistance in the Manzini-Mbabane corridor, Swaziland, in 2006. Antivir Ther **2008**; 13(Suppl 2):95–100.
22. Ministry of Health Malawi. Malawi integrated guidelines for providing HIV services. Available at: http://www.hivunitmohmw.org/Main/AntiretroviralTherapy. Accessed 25 March 2012.
23. Ministry of Health Malawi. Treatment of AIDS—guidelines for the use of antiretroviral therapy in Malawi. 3rd ed. Available at: http://www.hivunitmohmw.org/Main/AntiretroviralTherapy. Accessed 2 November 2011.
24. WHO. WHO recommendations for clinical mentoring to support scale-up of HIV care, antiretroviral therapy and prevention in resource-constrained settings. Available at: http://www.who.int/hiv/pub/meetingreports/clinicalmentoring/en/index.html. Accessed 2 November 2011.
25. Tweya H, Gareta D, Chagwera F, et al. Early active follow-up of patients on antiretroviral therapy (ART) who are lost to follow-up: the "Back-to-Care" project in Lilongwe, Malawi. Trop Med Int Health **2010**; 15(Suppl 1):82–9.
26. Kantor R, Katzenstein D. Drug resistance in non-subtype B HIV-1. J Clin Virol **2004**; 29:152–9.
27. Weigel R, Estill J, Egger M, et al. Mortality and loss to follow-up in the first year of ART: Malawi national ART programme. AIDS **2012**; 26:365–73.
28. Fox MP, Rosen S. Patient retention in antiretroviral therapy programs up to three years on treatment in sub–Saharan Africa, 2007–2009: systematic review. Trop Med Int Health **2010**; 15(Suppl 1):1–15.
29. Wadonda-Kabondo N, Myo K, Devos J, et al. Prevalence of HIV-1 drug resistance before treatment initiation and at 12 months after treatment initiation in four antiretroviral treatment sites in the Malawi ART program. Clin Infect Dis **2011**.
30. Hamers RL, Wallis CL, Kityo C, et al. HIV-1 drug resistance in antiretroviral-naive individuals in sub–Saharan Africa after rollout of antiretroviral therapy: a multicentre observational study. Lancet Infect Dis **2011**; 11:750–9.
31. Dagnra AY, Vidal N, Mensah A, et al. High prevalence of HIV-1 drug resistance among patients on first-line antiretroviral treatment in Lome, Togo. J Int AIDS Soc **2011**; 14:30.

32. Hosseinipour MC, van Oosterhout JJ, Weigel R, et al. The public health approach to identify antiretroviral therapy failure: high-level nucleoside reverse transcriptase inhibitor resistance among Malawians failing first-line antiretroviral therapy. AIDS **2009**; 23:1127–34.
33. El-Khatib Z, Ekstrom AM, Ledwaba J, et al. Viremia and drug resistance among HIV-1 patients on antiretroviral treatment: a cross-sectional study in Soweto, South Africa. AIDS **2010**; 24: 1679–87.
34. Oyugi JH, Byakika-Tusiime J, Charlebois ED, et al. Multiple validated measures of adherence indicate high levels of adherence to generic HIV antiretroviral therapy in a resource-limited setting. J Acquir Immune Defic Syndr **2004**; 36:1100–2.
35. Oyugi JH, Byakika-Tusiime J, Ragland K, et al. Treatment interruptions predict resistance in HIV-positive individuals purchasing fixed-dose combination antiretroviral therapy in Kampala, Uganda. AIDS **2007**; 21:965–71.
36. Veenstra N, Whiteside A, Gibbs A, et al. HEARD: unplanned ART interruptions in southern Africa: what can we do to minimise the long-term risks? Final Report **2010**. Available at: http://www.heard.org.za/downloads/unplanned-art-interruptions-policy-brief.pdf. Accessed 25 March 2012.