# Second-line treatment in the Malawi antiretroviral programme: high early mortality, but good outcomes in survivors, despite extensive drug resistance at baseline[*]

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## Abstract

**Objectives—**The Malawi antiretroviral therapy (ART) programme uses the public health approach to identify ART failure. Advanced disease progression may occur before switching to second-line ART. We report outcomes for patients evaluated and initiated on second-line treatment in Malawi.

**Methods—**Patients meeting Malawi immunological or clinical criteria for ART failure in two large urban ART clinics were evaluated for virological failure (viral load >400 HIV-1 RNA copies/mL) and, if failure was confirmed, initiated on second-line ART (zidovudine/lamivudine/ tenofovir/lopinavir/ritonavir). Patients were seen monthly and laboratory evaluations were performed quarterly and as needed. We performed logistic regression modelling to identify factors associated with mortality, mortality or new HIV illnesses, and virological suppression at 12 months.

**Results—**Of the 109 patients with confirmed virological failure, five patients died prior to initiation, three declined switching and 101 patients initiated second-line treatment. Over 12 months, 10 additional patients died, 34 patients experienced 45 HIV-related events, and 19 patients experienced grade 3 or 4 toxicities. Among survivors, 85.2% had HIV-1 RNA<400 copies/mL at

12 months. While power to distinguish differences was limited, response rates were similar regardless of baseline resistance level. The median CD4 count increase was 142 cells/μL. World Health Organization clinical failure at baseline [odds ratio (OR) 3.47; 95% confidence interval (CI) 1.14–10.59] and body mass index <18.5 (OR 4.43; 95% CI 1.15–17.12) were risk factors for death. Baseline CD4 count <50 cells/μL was associated with increased risk for death or morbidity at 12 months (OR 2.57; 95% CI 1.01–6.52).

**Conclusions—**Second-line treatment in Malawi was associated with substantial mortality, morbidity and toxicity but, among survivors, virological outcomes were favourable.

### Keywords

antiretroviral therapy; HIV resistance; Malawi; resource-limited setting; mortality; second line

## Introduction

The Malawi national antiretroviral therapy (ART) programme is implemented using the public health approach [1]. Patients start ART based mainly on World Health Organization (WHO) clinical staging, and the Malawi guidelines recommend switching therapy for failure determined by immunological or clinical criteria [2]. HIV-1 RNA monitoring is not a part of the ART programme. Since the free ART programme began in 2004, over 220 000 Malawians have been started on the standard first-line ART regimen, a fixed-dose combination of nevirapine (NVP), stavudine (d4T) and lamivudine (3TC) [3]. With the large population on treatment, regimen failure is inevitable in a substantial number of patients. Currently, the Malawi ART programme recommends a combination of zidovudine (ZDV), 3TC, tenofovir (TDF) and lopinavir/ritonavir (LPV/r) for those failing the first-line regimen [2,4]. The rationale of the three-nucleoside reverse transcriptase inhibitor (NRTI) backbone is to provide empirical coverage of accumulated mutations, given that failure will often be identified late as a result of the clinical and immunological monitoring strategy, on the assumption that 3TC may have residual activity, and that maintaining the M184 mutation increases the susceptibility of HIV to ZDV or TDF [5–8].

In Malawi, high levels of NRTI resistance are present when ART failure is detected using clinical and immunological criteria [9]. Approximately 17% of patients would be expected to have no fully active NRTI agents, even with the three-NRTI backbone. Similar paucity of active NRTI agents for second-line treatment has been noted in Thailand [10]. While LPV/r has been used successfully as monotherapy in ART-naïve populations [11,12], how failing patients will respond to an LPV/r-based second-line regimen with a suboptimal NRTI backbone has not been extensively studied.

To date, there are few data on the response to second-line treatment in resource-limited settings, particularly in the setting of confirmed extensive drug resistance. We aimed to document the response to second-line ART among Malawian patients with confirmed virological failure after identification by clinical and immunological means.

## Methods

### Study setting

This prospective cohort study was conducted at two urban sites in Lilongwe and Blantyre, Malawi, both following approximately 4000 patients on ART during the study period (January 2006 to July 2008). These were the only government clinics in Malawi with access to free second-line ART. Laboratory tests were performed at the University of North Carolina Research Project, Lilongwe and at the College of Medicine-Johns Hopkins Research Project, Blantyre.

### Population and design

Patients older than 13 years suspected of failing a standard first-line ART regimen consisting of NVP, or efavirenz in the case of previous NVP toxicity, 3TC and d4T, or ZDV in the case of previous d4T toxicity, were referred to the study teams. Patients were reviewed to confirm immunological failure (based on documented CD4 trends) and/or clinical failure (new or progressive WHO stage IV conditions). Patients with viral load >400 HIV-1 RNA copies/mL were defined as virological failures and those with low-level viraemia (400–1000 copies/mL) were confirmed prior to switching to second-line treatment. First-line therapy was maintained until the switch to second-line therapy occurred. All patients initiating second-line treatment within the public sector of the national ART programme at these centres during January 2006 to July 2007 were included in this study.

### Follow-up procedures

Patients were assessed monthly for toxicity, new WHO clinical stage 2, 3 or 4 events, and adherence through a short questionnaire and pill counts. HIV-1 RNA measurements (Roche Amplicor®; Roche, Basel, Switzerland; detection level 400 copies/mL), Complete Blood Count (CBC), CD4 cell counts [either FacsCount (Becton-Dickinson, Franklin Lakes, NJ, USA) or EPICS-MCL Pan-Leuco Gating method (Beckman Coulter, Brea, CA, USA)], liver function tests, and serum creatinine and random blood glucose measurements were performed at baseline and every 3 months or as clinically indicated. Genotype testing (TruGene HIV-1 Genotyping Kit; Siemens Medical Solutions Diagnostics, Tarrytown, NY, USA) on baseline samples was retrospectively performed for all patients with HIV-1 RNA>1000 copies/mL and was not available for clinical decision-making [9].

We managed TDF renal toxicity by substitution with abacavir (ABC), depending on availability; otherwise TDF was just discontinued. Patients with anaemia or neutropenia secondary to ZDV received either TDF/d4T/3TC or TDF/3TC. No substitute for LPV/r was available.

In the event of tuberculosis (TB) at failure identification, patients in Blantyre were maintained on first-line treatment until completion of TB treatment. In the case of incident TB during second-line treatment, ART was stopped. In Lilongwe, rifabutin was available and patients received rifabutin-based TB treatment concurrently with LPV/r-based ART.

### Ethical considerations

The study was approved by the Malawi National Health Sciences Research Committee and the University of North Carolina School of Medicine Committee for the protection of human subjects. Written informed consent was obtained from all patients.

### Outcome measurements

Outcomes included mortality, loss to follow-up (no visits within 90 days prior to or 30 days after the 12-month time-point) and any new WHO stage 2, 3 and 4 clinical events collected at each visit [1]. Laboratory evaluations were graded according to the division of AIDS (DAIDS) toxicity tables [13]. Creatinine clearance was calculated using the Cockcroft–Gault equation. We recorded any toxicity that led to treatment change, regardless of grade. The proportion of patients achieving HIV-1 RNA<400 copies/mL and the CD4 cell count was measured at 3, 6, 9 and 12 months. Cause of death was determined by chart review.

We evaluated adherence using the number of missed visits and the proportion of visits with no missed doses, and compared 'never missed' doses *vs.* 'ever missed' over the 12-month time period.

For resistance analysis, we categorized mutations according to the International AIDS Society USA (IAS-USA) recommendations [14] and categorized patients according to the number of active NRTI drugs based on the baseline genotype pattern. Those with only M184V and NNRTI mutations or wild-type virus were considered to have at least two fully active NRTI drugs or 'low' resistance; patients with any thymidine analog mutations (TAMs) or K65R/70E or Q151M were considered to have at least one fully active NRTI drug or 'medium' resistance; and patients with the 69 insertion or Q151M complex in combination with K65R or K70E were considered to have no active NRTI drugs or 'high' resistance. Additionally, we evaluated responses in patients with wild-type virus, any TAMs, and at least three TAMs.

### Statistical considerations

In all analyses, STATA v.10 (STATA Corp., College Station, TX, USA) was used. Student's $t$-test and the $\chi^2$ or Fisher's exact test were used to compare continuous and categorical variables, respectively.

We performed logistic regression analysis to identify factors associated with mortality, mortality and/or morbidity (new WHO stage 3 or 4 clinical event) at 6 and 12 months, and virological suppression to HIV-1 RNA<400 copies at 12 months. For the mortality, and mortality and/or morbidity models, all confirmed first-line ART virological failures were included; however, for the virological suppression model, only those initiating second-line treatment were included. For all models, factors considered included age, gender, means of failure identification (any clinical *vs.* immunological only), HIV-1 RNA and CD4 cell count at time of failure identification, duration of first-line ART before presentation, haemoglobin and body mass index (BMI). Additionally, adherence measures (self report of ever having missed a dose/not having missed a dose) and degree of baseline resistance were included as factors in the model related to virological suppression. Categories for continuous variables

(age, CD4 cell count, HIV-1 RNA, duration on ART, BMI and haemoglobin) were chosen for clinical significance and to be consistent with the previous literature.

For the HIV suppression model, we employed intent-to-treat analysis with deaths and loss to follow-up, but not treatment switches because of toxicity, considered as failures. Model diagnostics were performed and, in the event of significant collinearity between variables, only one was included in the final multivariate model.

## Results

Among the approximately 8000 ART patients currently in follow-up and 54 external referrals, we evaluated 203 patients for suspicion of treatment failure based on clinical and immunological criteria (Fig. 1). Of these, 109 patients were recommended for switch to second-line ART after confirmation of virological failure. Five patients died prior to second-line ART initiation (Figs 1 and 2) with a median time between screening and death of 19 days (range 7–24 days). Three patients declined switching in the government clinics and were excluded from follow-up analysis.

Patients initiating second-line treatment ($n = 101$) had a median [interquartile range (IQR)] CD4 count of 65 (22–173) cells/μL and HIV-1 RNA of 52 939 (15 739–148149) copies/mL (Table 1). As previously described [9], the population had extensive baseline resistance mutations to the NRTI class of drugs (Table 1), but no patient had any mutations associated with LPV/r resistance.

### Clinical outcomes and clinical progression

Among 101 patients who initiated second-line treatment, 10 patients (10%) died during the 12 months of follow-up (Fig. 2). All deaths occurred in the first 6 months of treatment, with six deaths in the first 3 months post initiation.

Primary causes of death among patients with confirmed virological failure ($n = 106$) included: Kaposi sarcoma (KS) (four patients), TB (two), sepsis (two), wasting syndrome (one), anaemia (one) and other (five). Three patients were lost to follow-up between 6 and 12 months. HIV-related illnesses were common during the follow-up period. Thirty-four patients experienced 45 HIV-related events during the 12 months after the initiation of second-line treatment, and 69% of events occurred in the first 6 months. Events included bacterial pneumonia [13], KS progression [11], TB (seven), oral candidiasis (nine), sepsis (two) and progressive cryptococcal meningitis (three).

Overall, 15 patients required TB treatment either at initiation (eight patients) or during second-line treatment (seven patients). Eight patients completed rifabutin-based treatment, and one died before initiating the rifabutin-based treatment. Six received rifampicin-based treatment before initiation of second-line ART, of whom one died prior to commencing second-line ART.

On multivariate analysis, clinical failure as the indicator of first-line failure and BMI<18.5 were independent risk factors of death at 12 months among all virologically confirmed

patients ($n$ = 106) (Table 2). At both 6 and 12 months, CD4<50 cells/μL was independently associated with death and morbidity (Table 2).

## Toxicity

Twenty-eight grade 3 or 4 toxicities occurred in 19 individuals after second-line ART initiation. These included haemoglobin <7.5 mg/dL (nine cases), absolute neutrophil count <750 cells/μL (11), creatinine >2.3 mg/dL (three), creatinine clearance <50 mL/min (15), glucose >251 mg/dL (three), and lactate >3.5 mmol/L (two). ZDV was discontinued because of either anaemia or neutropenia in seven patients. In four subjects with renal toxicity, TDF was substituted with ABC, and in one case of lactic acidosis all NRTIs including TDF were discontinued. LPV/r was not discontinued because of toxicity in any patient.

## Adherence

Among patients initiating treatment, 55% reported never missing a dose throughout the study period. Likewise, 55% of patients never missed a clinic visit but 29% of patients missed one visit, 11% missed two visits, and 5% missed three visits.

## Immunological and virological outcomes

Among survivors, the median increase in CD4 count was 142 cells/μL (IQR 66, 263) at 12 months and 85% of these patients had HIV-1 RNA<400 copies/mL at 12 months (Fig. 3). Overall, 75% of the 101 patients who started second-line therapy survived and were suppressed (Fig. 3). Of the 13 patients who had HIV-1 RNA>400 copies/mL at month 12, six were never suppressed and seven had initial suppression but rebounded.

On treatment, the HIV-1 RNA suppression rate for patients with wild-type virus was 60% [95% confidence interval (CI) 15–95%] compared with 94% (95% CI 87–100%) for patients with any TAMs and 95% (95% CI 85–100%) for those with at least three TAMs. HIV-1 RNA suppression rates varied according to the number of active NRTI drugs: at least two active drugs (low), 71% (95% CI 50–93%); one active drug (medium), 92% (95% CI 85–100%); and no active drugs (high), 97% (95% CI 77–100%). Adherence rates (never missed doses) were 48% for those with at least two active drugs (low), 59% for those with one active drug (medium), and 56% for those with no active drugs (high) ($P$ = 0.7), which corresponded to HIV-1 RNA suppression rates of 90% for those with at least two active drugs (low), 96% for those with one active drug (medium), and 89% for those with no active drugs (high) ($P$ = 0.6). Among patients who ever missed doses, HIV-1 RNA suppression rates were 55% for those with at least two active drugs (low), 84% for those with one active drug (medium), and 85% for those with no active drugs (high) ($P$ = 0.15).

Factors associated with HIV-1 RNA>400 copies/mL at 12 months on univariate analysis included having a presenting CD4 count <50 cells/μL and HIV-1 RNA > 100 000 copies/mL (Table 3). Paradoxically, having extensive baseline resistance resulted in better virological suppression (Table 3). However, on multivariate analysis, only poor adherence (ever missing a dose) remained statistically significant. Duration on first-line treatment >3 years was not associated with increased risk of failure.

## Discussion

In our cohort of ART failure patients identified by clinical and immunological criteria in the public health setting who were confirmed to have virological failure, there is substantial early mortality on second-line ART. Identification of failure by clinical criteria, in particular, was associated with an increased risk of death in the first 6 months as well as new and progressive HIV-associated illnesses. However, among survivors, high rates of virological suppression and good immunological recovery occurred despite extensive baseline resistance. As with most ART studies, we found adherence to be a significant factor in the successful suppression of HIV-1 RNA.

Early mortality upon initiation of first-line ART has been well described in resource-limited settings [15–18] and predictors for mortality have consistently included low BMI, low CD4 cell count and anaemia [15–19]. Low BMI and low CD4 cell counts were similarly associated with early HIV-associated illnesses and mortality in our cohort of patients initiating second-line ART and in a study of second-line ART patients treated in Médecins Sans Frontières (MSF) programmes [20]. Additionally, the mortality prior to initiation of second-line ART mirrors mortality before initiation of first-line treatment [21–23]. The striking similarity of severe events occurring around initiation of first- and second-line ART in resource-limited settings suggests that the advanced stage of HIV disease of patients in both situations is the underlying cause. The primary reason why our ART failure patients were in such advanced stages of disease is likely to be the reliance on clinical and, less consistently, immunological monitoring for identification of ART failure in the Malawi ART programme. The results from our second-line programme may improve over time if clinicians become more experienced in identifying ART failure, and if second-line ART becomes more widely available outside tertiary centres, avoiding lengthy referral procedures.

We have previously demonstrated a complex array of mutation patterns in this patient cohort such that 17% of patients would be expected to have no active NRTI in the second-line regimen and an additional 68% would have reduced susceptibility to the NRTI combination [9]. Despite the degree of baseline resistance, among our survivors, there was a high rate of virological suppression and immunological recovery, comparable to findings in other studies in both resource-rich and resource-poor settings [20,24]. While our study was not powered to detect differences in suppression rates according to varying degrees of resistance, we found the presence of more complex resistance genotypes was actually associated with more successful suppression on univariate analysis. The presence of the wild type or only low genetic barrier resistance mutations (M184V or NNRTI mutations) may suggest recent nonadherence as the aetiology of failure for both first- and second-line regimens, whereas more highly adherent patients, paradoxically, may have accumulated more mutations in response to their failing first-line combination. Alternatively, if patients were recently nonadherent, we may have failed to detect minority variants that would suggest high resistance. LPV/r has been shown to be an effective monotherapy regimen in ART-naïve patients in both the short and longer term [11,12]. The successful suppression of HIV-1 RNA in the setting of known extensive genotypic NRTI resistance suggests potential for its use as monotherapy after first failure of an NNRTI-based regimen also. Alternatively, residual

NRTI activity may be underestimated by genotype and phenotype testing [5,6,8,25]. Longer term follow-up will be required to determine the durability of our findings.

Drug toxicity and drug substitutions were common in our study, underscoring the need for laboratory capacity in settings where second-line treatment is available. In particular, renal toxicity to TDF was somewhat higher than reported in series of first-line treatment of similar treatment duration [26,27]. LPV/r has recently been shown to increase TDF concentrations [28] and this may explain our findings, although this hypothesis is controversial [29,30]. Additionally, ZDV-induced anaemia required frequent substitutions. While genotypic and phenotypic resistance results theoretically supported the use of ZDV/3TC/TDF in second-line treatment [9], the high rates of HIV-1 RNA suppression in patients with the most extensive NRTI resistance suggest that the NRTI backbone may unnecessarily complicate patient management by frequently inducing toxicity rather than improve virological outcome when used in all patients in the absence of prospective resistance testing. Using three NRTIs in all patients also increases overall costs. Further studies to determine optimal second-line regimens for resource-limited settings are urgently needed.

TB was common in our study population. Malawi follows WHO guidelines for the treatment of TB with a 6-month rifampicin-containing regimen, which results in a delay or interruption of LPV/r-based second-line ART until completion of the TB treatment, with the associated risks of severe morbidity and mortality. Strategies to overcome the unfavourable pharmacokinetics have not been successful [31–33], or have led to potentially dangerous hepatotoxicity [34]. Rifabutin-based TB treatment, compatible with protease inhibitor therapy, has limited availability and experience in its use in resource-limited settings is small. We observed successful treatment in all patients we treated with the rifabutin-based combination. The addition of rifabutin to the WHO essential drugs list should improve availability [35] and allow more successful treatment of both HIV and TB in patients on second-line ART.

Given the monitoring strategy used in Malawi, we can assume that a large number of virological failure cases were not identified. Within the national programme, as of December 2008, only 518 (0.3%) of the 145 479 patients known to be alive and on ART had been switched to a second-line regimen [3], underscoring the low identification of virological failure nationally. We enrolled all consecutive patients beginning second-line treatment at both clinics and thus our findings are representative of the treatment outcomes that would be expected in an ART programme following a public health approach. Our study is among the first descriptions of virological outcomes of second-line treatment in resource-limited settings linked to baseline resistance data. While our suppression rates are promising, longer duration of follow-up is required.

Our data add to the ongoing debate regarding the optimal way to identify and manage ART failure in resource-limited settings. Increasing evidence demonstrates the poor predictive value of clinical and immunological definitions of ART failure and the need for viral load testing to accurately identify failure [36–38]. Moreover, accumulation of resistance mutations with its potentially compromised treatment responses and risk of transmission of resistant virus have also prompted calls for earlier failure detection potentially through

HIV-1 RNA monitoring [9,10]. Computer modelling of ART outcomes in the setting of limited treatment options suggests that virological monitoring will have minimal impact on long-term survival [39]. Yet, in a recent home-based care clinical trial in rural Uganda, clinical monitoring was associated with an increased risk of death or AIDS-defining event at 3 years [40]. Additionally, the Development of Antiretroviral Therapy in Africa (DART) study confirmed that clinical monitoring alone was associated with a small but significant increase in the risk of death and AIDS progression compared with quarterly CD4 cell count monitoring [41] but cost effective analysis suggested quarterly CD4 monitoring was not cost effective at current prices [42].

Somewhat surprisingly, we demonstrated that extensive NRTI resistance did not adversely affect second-line virological and immunological outcomes over a year of follow-up. However, we observed substantial, primarily early, mortality and a large proportion of survivors experienced new or recurrent WHO stage 3 or 4 illnesses. Our observations argue strongly for earlier detection of ART failure, either by a more sensitive clinical/ immunological algorithm or by point-of-care HIV-1 viral load monitoring. Resistance testing, while potentially useful, is still very expensive and may be less important for the individual patient. The poor response rate in those individuals with the most limited resistance and the association of virological failure with nonadherence remind us of the importance of adherence in all settings in which ART is administered.

## Acknowledgments

## References

1. , editor. World Health Organization. Antiretroviral Therapy for HIV Infection in Adults and Adolescents in Resource-Limited Settings: Toward Universal Access. Recommendations for a Public Health Approach. Geneva: World Health Organization; 2006. p. 128

2. , editor. Ministry of Health Malawi. Treatment of AIDS: Guidelines for the Use of Antiretroviral Therapy in Malawi. 2. Lilongwe: Malawi Ministry of Health; 2006. p. 155

3. Department of HIV and AIDS Ministry of Health. Department of HIV and AIDS MoH. Quarterly Report of the Antiretroviral Treatment Programme in Malawi with Results up to 31st December 2008. Lilongwe: Malawi Ministry of Health; 2008.

4. Ministry of Health Malawi. Treatment of AIDS: Guidelines for the Use of Antiretroviral Therapy in Malawi. 3. Lilongwe: Malawi Ministry of Health; 2008.

5. Eron JJ Jr, Bartlett JA, Santana JL, et al. Persistent antiretroviral activity of nucleoside analogues after prolonged zidovudine and lamivudine therapy as demonstrated by rapid loss of activity after discontinuation. J Acquir Immune Defic Syndr. 2004; 37:1581–1583. [PubMed: 15577413]

6. Castagna A, Danise A, Menzo S, et al. Lamivudine monotherapy in HIV-1-infected patients harbouring a lamivudine-resistant virus: a randomized pilot study (E-184V study). AIDS. 2006; 20:795–803. [PubMed: 16549962]

7. Larder BA, Kemp SD, Harrigan PR. Potential mechanism for sustained antiretroviral efficacy of AZT-3TC combination therapy. Science. 1995; 269:696–699. [PubMed: 7542804]

8. Campbell TB, Shulman NS, Johnson SC, et al. Antiviral activity of lamivudine in salvage therapy for multidrug-resistant HIV-1 infection. Clin Infect Dis. 2005; 41:236–242. [PubMed: 15983922]

9. 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 (London, England). 2009; 23(9):1127–1134.

10. 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; 44:447–452. [PubMed: 17205457]

11. Arribas JR, Pulido F, Delgado R, et al. Lopinavir/ritonavir as single-drug therapy for maintenance of HIV-1 viral suppression: 48-week results of a randomized, controlled, open-label, proof-of-concept pilot clinical trial (OK Study). J Acquir Immune Defic Syndr. 2005; 40:280–287. [PubMed: 16249701]

12. Pulido F, Delgado R, Perez-Valero I, et al. Long-term (4 years) efficacy of lopinavir/ritonavir monotherapy for maintenance of HIV suppression. J Antimicrob Chemother. 2008; 61:1359–1361. [PubMed: 18343802]

13. Division of AIDS National Institutes of Health. [accessed 16 June 2009] Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events. 2004. Available at www3.niaid.nih.gov/LabsAndResources/resources/DAIDSClinRsrch/PDF/DAIDSAEGradingTable.pdf

14. Johnson VA, Brun-Vezinet F, Clotet B, et al. Update of the drug resistance mutations in HIV-1. Top HIV Med. 2008; 16:138–145. [PubMed: 19106428]

15. Braitstein P, Brinkhof MW, Dabis F, et al. Mortality of HIV-1-infected patients in the first year of antiretroviral therapy: comparison between low-income and high-income countries. Lancet. 2006; 367:817–824. [PubMed: 16530575]

16. Stringer JS, Zulu I, Levy J, et al. Rapid scale-up of antiretroviral therapy at primary care sites in Zambia: feasibility and early outcomes. JAMA. 2006; 296:782–793. [PubMed: 16905784]

17. Ferradini L, Jeannin A, Pinoges L, et al. Scaling up of highly active antiretroviral therapy in a rural district of Malawi: an effectiveness assessment. Lancet. 2006; 367:1335–1342. [PubMed: 16631912]

18. Severe P, Leger P, Charles M, et al. Antiretroviral therapy in a thousand patients with AIDS in Haiti. N Engl J Med. 2005; 353:2325–2334. [PubMed: 16319381]

19. Lawn SD, Harries AD, Anglaret X, Myer L, Wood R. Early mortality among adults accessing antiretroviral treatment programmes in sub-Saharan Africa. AIDS (London, England). 2008; 22:1897–1908.

20. Pujades-Rodriguez M, O'Brien D, Humblet P, Calmy A. Second-line antiretroviral therapy in resource-limited settings: the experience of Médecins Sans Frontières. AIDS. 2008; 22:1305–1312. [PubMed: 18580610]

21. Hosseinipour MC, Neuhann FH, Kanyama CC, et al. Lessons learned from a paying antiretroviral therapy service in the public health sector at Kamuzu Central Hospital, Malawi: 1-year experience. J Int Assoc Phys AIDS Care (Chicago, IL). 2006; 5:103–108.

22. Lawn SD, Myer L, Orrell C, Bekker LG, Wood R. Early mortality among adults accessing a community-based antiretroviral service in South Africa: implications for programme design. AIDS (London, England). 2005; 19:2141–2148.

23. Fairall LR, Bachmann MO, Louwagie GM, et al. Effectiveness of antiretroviral treatment in a South African program: a cohort study. Arch Intern Med. 2008; 168:86–93. [PubMed: 18195200]

24. Madruga JV, Berger D, McMurchie M, et al. Efficacy and safety of darunavirritonavir compared with that of lopinavirritonavir at 48 weeks in treatment-experienced, HIV-infected patients in TITAN: a randomised controlled phase III trial. Lancet. 2007; 370:49–58. [PubMed: 17617272]

25. Deeks SG, Hoh R, Neilands TB, et al. Interruption of treatment with individual therapeutic drug classes in adults with multidrug-resistant HIV-1 infection. J Infect Dis. 2005; 192:1537–1544. [PubMed: 16206068]

26. Gallant JE, DeJesus E, Arribas JR, et al. Tenofovir DF, emtricitabine, and efavirenz vs. zidovudine, lamivudine, and efavirenz for HIV. N Engl J Med. 2006; 354:251–260. [PubMed: 16421366]

27. Gallant JE, Staszewski S, Pozniak AL, et al. Efficacy and safety of tenofovir DF vs stavudine in combination therapy in antiretroviral-naive patients: a 3-year randomized trial. JAMA. 2004; 292:191–201. [PubMed: 15249568]

28. Kearney BP, Mathias A, Mittan A, Sayre J, Ebrahimi R, Cheng AK. Pharmacokinetics and safety of tenofovir disoproxil fumarate on coadministration with lopinavir/ritonavir. J Acquir Immune Defic Syndr. 2006; 43:278–283. [PubMed: 17079992]

29. Moore, R.; Gallant, J. Renal Function after Use of Tenofovir as Part of the Initial ART Regimen. ICAAC/IDSA; Washington, DC: 2008.

30. Short, WR.; Solari, P.; Kaplan, B. Comparison of Renal Function on NNRTI vs. Boosted PI Based Tenofovir Based Regimens. ICAAC/IDSA; Washington, DC: 2008.

31. Mallolas J, Sarasa M, Nomdedeu M, et al. Pharmacokinetic interaction between rifampicin and ritonavir-boosted atazanavir in HIV-infected patients. HIV Med. 2007; 8:131–134. [PubMed: 17352770]

32. Ribera E, Azuaje C, Lopez RM, et al. Pharmacokinetic interaction between rifampicin and the once-daily combination of saquinavir and low-dose ritonavir in HIV-infected patients with tuberculosis. J Antimicrob Chemother. 2007; 59:690–697. [PubMed: 17307771]

33. la Porte CJ, Colbers EP, Bertz R, et al. Pharmacokinetics of adjusted-dose lopinavirritonavir combined with rifampin in healthy volunteers. Antimicrob Agents Chemother. 2004; 48:1553–1560. [PubMed: 15105105]

34. Nijland HM, L'Homme RF, Rongen GA, et al. High incidence of adverse events in healthy volunteers receiving rifampicin and adjusted doses of lopinavir/ritonavir tablets. AIDS. 2008; 22:931–935. [PubMed: 18453852]

35. World Health Organization. [accessed 6 July 2009] 17th Expert Committee on the Selection and Use of Essential Medicines. 2009. Available at www.who.int/selection_medicines/committees/expert/17/en/index.html

36. van Oosterhout JJ, Brown L, Weigel R, et al. Diagnosis of antiretroviral therapy failure in Malawi: poor performance of clinical and immunological WHO criteria. Trop Med Int Health. 2009; 14(8):856–861. [PubMed: 19552661]

37. Mee P, Fielding KL, Charalambous S, Churchyard GJ, Grant AD. Evaluation of the WHO criteria for antiretroviral treatment failure among adults in South Africa. AIDS. 2008; 22:1971–1977. [PubMed: 18784460]

38. Reynolds SJ, Nakigozi G, Newell K, et al. Failure of immunologic criteria to appropriately identify antiretroviral treatment failure in Uganda. AIDS (London, England). 2009; 23:697–700.

39. Phillips AN, Pillay D, Miners AH, Bennett DE, Gilks CF, Lundgren JD. Outcomes from monitoring of patients on antiretroviral therapy in resource-limited settings with viral load, CD4 cell count, or clinical observation alone: a computer simulation model. Lancet. 2008; 371:1443–1451. [PubMed: 18440426]

40. Coutinho, A.; Mermin, J.; Ekwaru, JP., et al. Utility of routine viral load, CD4 cell count, and clinical monitoring among HIV-infected adults in Uganda: a randomized trial. 15th Conference on Retroviruses and Opportunistic Infections; Boston, MA. 2008; Abstract 125

41. Mugyenyi, P.; Walker, S.; Hakim, J., et al., editors. Impact of routine laboratory monitoring over 5 years after antiretroviral therapy (ART) initiation on clinical disease progression of HIV-infected African adults: the DART Trial final results; 5th Conference on HIV Pathogenesis Treatment and Prevention; Cape Town, South Africa: International AIDS Society; 2009. Abstract TUSS102

42. Lara, AM.; Kigozi, J.; Amurwon, J., et al., editors. Cost Effectiveness analysis of routine laboratory or clinically driven strategies for monitoring antiretroviral therapy in Uganda and Zimbabwe (DART trial); 5th Conference on HIV Pathogenesis Treatment and Prevention; Cape Town, South Africa: International AIDS society; 2009. Abstract TUSS103
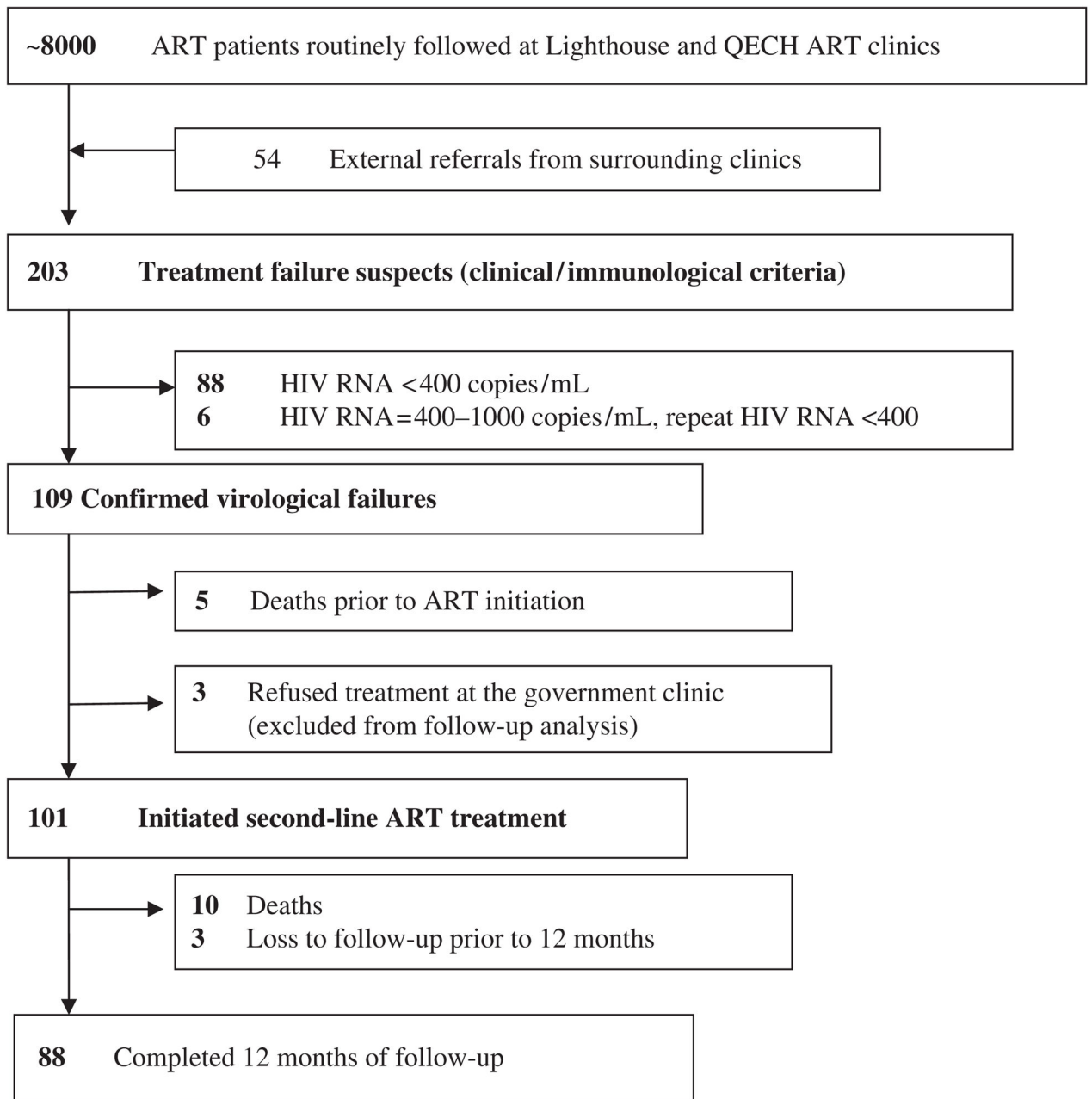
~8000 ART patients routinely followed at Lighthouse and QECH ART clinics

54 External referrals from surrounding clinics

203 Treatment failure suspects (clinical/immunological criteria)

88 HIV RNA <400 copies/mL
6 HIV RNA=400–1000 copies/mL, repeat HIV RNA <400

109 Confirmed virological failures

5 Deaths prior to ART initiation

3 Refused treatment at the government clinic
(excluded from follow-up analysis)

101 Initiated second-line ART treatment

10 Deaths
3 Loss to follow-up prior to 12 months

88 Completed 12 months of follow-up

**Fig. 1.**
Patient recruitment and flow chart. ART, antiretroviral therapy; QECH, Queen Elizabeth
Central Hospital; HIV, human immunodeficiency virus; RNA, ribonucleic acid.
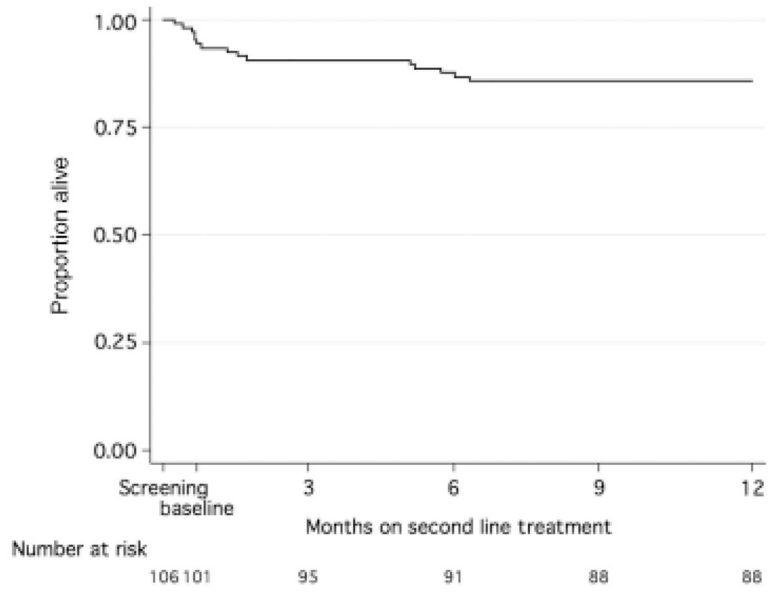
**Fig. 2.**
Kaplan–Meier estimates of proportion of patients with virologically confirmed failure (*n* = 106) who were alive after 12 months post identification of failure.
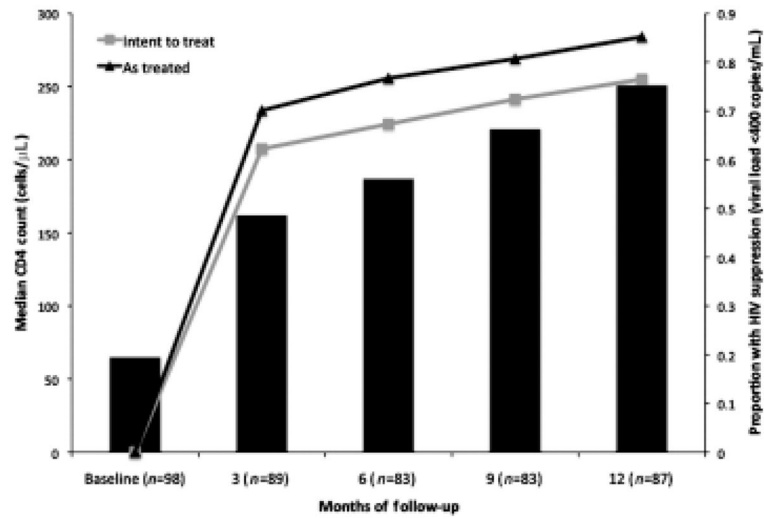
**Fig. 3.**
Trends in HIV-1 RNA suppression (<400 copies/mL) and median CD4 cell counts after initiation of second-line antiretroviral therapy to month 12. The lighter line represents the intent-to-treat analysis (death/lost = failure) and the darker line represents the as-treated analysis (death/lost = excluded). Solid bars represent median CD4 cell counts (death/lost = excluded).

**Table 1**

Demographics and clinical characteristics of patients at confirmed virological failure ($n = 109$) and on initiation of second-line antiretroviral therapy (ART) ($n = 101$)

| | Median (IQR) or % | |
|---|---|---|
| | At virological failure ($n = 109$) | On initiation of second-line ART ($n = 101$) |
| Age (years) | 37.5 (31.5–46.0) | 38.0 (32.0–46.0) |
| Per cent female | 54% | 55% |
| Per cent male | 46% | 46% |
| Time on first-line line ART (months) | 34.2 (24.5–48.0) | 35.2 (25.4–49.0) |
| CD4<50 cells/μL | 44% | 46% |
| 50 CD4 200 cells/μL | 34% | 34% |
| CD4>200 cells/μL | 22% | 21% |
| Body mass index | 21.0 (18.4–23.5) | 21.2 (18.5–23.6) |
| Haemoglobin (g/dL) | 12.4 (10.7–13.9) | 12.3 (10.6–13.9) |
| Clinical failure | 6% | 8% |
| Immunological failure | 76% | 73% |
| Both clinical and immunological failure | 19% | 18% |
| Active tuberculosis | 16% | 14% |
| Kaposi sarcoma | 11% | 11% |
| Resistance profile (%)[*] | | |
| Wild type (no mutations) | | 5% |
| NNRTI ± M184V mutations only | | 17% |
| Any TAMs | | 56% |
| 3 TAMs | | 25% |
| K65R or K70E | | 24% |
| K65R/70E 1Q151M or 69 insertion | | 17% |

[*]Resistance testing was only performed on patients initiating second-line ART with a sufficient sample ($n = 96$, with two nonamplified samples). Detailed resistance data are available [9].

IQR, interquartile range; NNRTI, nonnucleoside reverse transcriptase inhibitor; TAMs, thymidine analog mutations.

**Table 2**

Multivariate analysis evaluating factors associated with death at 6 and 12 months, and death or morbidity at 6 and 12 months, for all patients identified as having confirmed virological failure ($n = 106$)

| Baseline factor | Death by 6 or 12[*] months adjusted OR (95% CI) | Death or morbidity by 6 months adjusted OR (95% CI) | Death or morbidity by 12 months adjusted OR (95% CI) |
|---|---|---|---|
| Clinical failure | **3.47 (1.14–10.59)** | 2.76 (0.85–8.95) | 1.51 (0.49–4.67) |
| CD4<50 cells/μL | 1.09 (0.37–3.27) | **3.13 (1.05–9.31)** | **2.57 (1.01–6.52)** |
| HIV RNA>100 000 copies/mL | 1.10 (0.35–3.50) | 0.78 (0.24, 2.59) | 1.34 (0.49–3.67) |
| Body mass index <18.5 m$^2$/kg | **4.43 (1.15–17.12)** | 1.99 (0.67–5.86) | 1.82 (0.66–5.00) |
| Haemoglobin <10 g/dL | 2.97 (0.87–10.08) | 3.60 (0.97–13.3) | 1.82 (.056, 5.91) |
| Duration of ART>3 years prior to identified failure | 1.74 (0.37, 8.24) | 2.10 (0.69–6.36) | 0.46 (0.20, 1.03) |

Values in bold denote statistical significance.

[*]No deaths occurred after 6 months therefore analysis at 6 and 12 months is identical.

ART, antiretroviral therapy; CI, confidence interval; OR, odds ratio; HIV, human immunodeficiency virus; RNA, ribonucleic acid.

**Table 3**

Univariate and multivariate analysis evaluating factors associated with failure to achieve HIV RNA<400 copies/mL at 12 months in patients initiating second-line antiretroviral therapy (ART) ($n$ = 101)

| Predictor | OR (95% CI) | aOR (95% CI) |
|---|---|---|
| Age <18 years | 3.96 (0.67, 13.0) | 1.63 (0.15, 17.2) |
| Female gender | 2.00 (0.57, 7.00) | 5.92 (0.95, 36.76) |
| ART>3 years | 0.31 (0.08, 1.20) | 0.50 (0.10, 2.42) |
| CD4<50 cells/μL | **8.6 (2.40, 30.78)** | 1.34 (0.17, 10.81) |
| 50 CD4 200 cells/μL | 2.10 (0.67, 6.49) | 1.06 (0.14, 8.19) |
| CD4>200 cells/μL | 1.0 | 1.0 |
| HIV RNA>100 000 copies/mL | **3.47 (1.08, 11.09)** | 0.79 (0.15, 4.19) |
| WHO clinical failure | **11.54 (1.48, 90.21)** | 1.33 (0.24, 7.44) |
| WHO immunological failure | **54.21 (14.5, 202.7)** | |
| Resistance profile | | |
| 2 active NRTIs ('low') | 1.0 | |
| 1 active NRTI ('medium') | 0.45 (0.16, 1.2) | |
| No active NRTIs ('high') | **0.17 (0.03, 0.94)** | |
| Ever missed a dose/not | 3.15 (0.90, 11.05) | **5.70 (1.16, 27.93)** |
| Number of missed visits | **2.50 (1.35, 4.63)** | |
| Haemoglobin <10 g/dL | 0.82 (0.16, 4.11) | 1.15 (0.17, 7.87) |
| Body mass index <18.5 | 2.75 (0.85, 8.85) | 2.09 (0.33, 13.30) |
| Tuberculosis diagnosis (baseline or during study) | 1.09 (0.21, 5.54) | 0.86 (0.08, 9.36) |

Values in bold denote statistical significance.

WHO immunological failure dropped for the multivariate analysis because of collinearity with CD4 cell count categories; the number of missed visits decreased for the multivariate analysis because of collinearity with ever missed a dose/not. The resistance profile decreased in the multivariate analysis because of missing values ($n$ = 7).

aOR, adjusted odds ratio; NRTI, nucleoside reverse transcriptase inhibitor; OR, odds ratio; WHO, World Health Organization.