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Title:

Virological failure, HIV-1 drug resistance and early mortality in adults admitted to hospital in Malawi: an observational cohort study

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Abstract

Background: Antiretroviral therapy (ART) scale-up in sub-Saharan Africa (SSA) combined with weak routine virological monitoring has driven increasing HIV drug resistance. We investigated ART failure, drug resistance and early mortality among hospital inpatients in Malawi.

Methods: An observational cohort study nested in a trial of urine-based TB screening in unselected HIV-positive adults followed up for 56-days. Patients taking ART for ≥6 months at hospital admission had frozen plasma samples tested for HIV-1 viral load. Those with HIV-1 RNA ≥1000 copies per mL had drug resistance testing by ultra-deep sequencing, with drug resistance defined as intermediate or high-level resistance using the Stanford HIVDR Algorithm

Findings: Of 1316 patients recruited in the Malawi trial site between October 2015 and September 2017, 786 had taken ART for ≥6 months of whom 252/786 (32.1%) patients had viral load ≥1000 copies per mL (virological failure). Mean age was 38 years, 61.5% were female and median CD4 count was 60 cells/μL. Of 237 (94.0%) patients with HIV drug resistance results available, 195 (82.3%) had resistance to lamivudine, 128 (54.0%) to tenofovir and 219 (92.4%) to efavirenz. Resistance to at least 2 drugs was common (196/237, 82.7%) and this was associated with increased 2-month mortality (adjusted hazard ratio 1.7, 95% CI 1.2-2.4, p=0.004).

Interpretation: Interventions are urgently needed and should target ART clinic, hospital and post-hospital care, including differentiated care focusing on patients with advanced HIV, rapid viral load testing and routine access to drug resistance testing. Prompt diagnosis and switching to alternative ART could reduce early mortality among HIV-positive inpatients.

Funding: Joint Global Health Trials Scheme of the Medical Research Council, the UK Department for International Development, and the Wellcome Trust.

Research in Context

Evidence before this study: We searched MEDLINE for studies that investigated virological failure and/or HIV drug resistance in HIV-positive patients on antiretroviral therapy (ART) who are hospital inpatients in Africa, published between Jan 1, 2005, (ART was not in widespread use prior to this), to Jan 1 2020. We combined search terms for HIV ("HIV", "HIV-1", "Human immunodeficiency virus" or "AIDS") with terms for virological failure ("virological failure", "viral failure", "failure", "antiretroviral failure", "treatment failure" or "ART failure") or resistance ("resistance", "drug resistance", "HIV resistance", "antiretroviral resistance", or "ART resistance") with terms for hospital inpatient ("hospital", "inpatient", "in-patient", "admission" or "hospitalised"), and Africa. We only identified 1 observational cohort study that reported prevalence of virological failure in two cohorts of inpatients. This study showed high prevalence of patients who were already taking ART, but had HIV-1 viral load ≥1000 copies per mL. However, the study did not do viral load testing on all patients taking ART, introducing bias. There were no studies reporting HIV drug resistance among hospital inpatients. Other studies identified all reported data from outpatient clinics.

Added value of this study: We performed HIV viral load testing on unselected patients admitted to hospital who were taking ART for at least 6 months, and looked for HIV drug resistance mutations for patients who had high HIV viral loads. We then assessed whether the presence of drug resistance was associated with mortality. Approximately one-third of inpatients taking ART had virological failure, and most of these patients had drug resistance to first-line ART which was associated with increased early mortality.

Implications of all the evidence: ART failure and drug resistance is a major problem in hospital inpatients in high HIV prevalent settings. The evidence supports testing hospital inpatients for ART failure, ideally using a rapid HIV-1 viral load assays so results are available quickly and can be immediately acted upon. The high prevalence of drug resistance and association with early mortality suggest these patients should be switched to alterative ART, and supports development of low-cost, rapid assays to detect HIV drug resistance. Differentiated ART clinic care to support adherence and detect ART failure in advanced HIV, testing and screening for opportunistic infections and improved post discharge care may improve outcomes, although further evidence for such interventions will be needed.

Introduction

Despite the unprecedented scale-up of antiretroviral therapy (ART) in high-HIV prevalence settings in sub-Saharan Africa (SSA), HIV remains a common cause of admission to hospital, with high early mortality (31% in the African region).[1,2] Whilst older cohorts of HIV-positive patients from SSA were predominately newly diagnosed or ART naïve, more recent data suggests that patients admitted with advanced HIV (defined by World Health Organization [WHO] as CD4 count <200 cells/µL or stage 3 or 4 illness) are mostly ART experienced.[3–6]

HIV drug resistance is increasingly common in SSA, with recent estimates of 10-15% prevalence for non-nucleoside reverse transcriptase inhibitors (NNRTIs) in untreated patients (transmitted drug resistance), and much higher rates (50-80% for NNRTIs) in patients failing ART.[7–9] Laboratory and clinical capacity within SSA remains limited, however, with most countries managing patients on ART with infrequent viral load testing and minimal access to drug resistance testing. As distinguishing HIV drug resistance from alternative explanations for progressive illness on ART is usually not possible, patients who develop advanced HIV while established on ART either are assumed to have treatment failure or go undetected.

Although most available data on HIV drug resistance come from outpatient clinics, inpatients represent a key target group for intensified interventions given their relatively high risks of treatment failure, advanced immunosuppression and high short-term mortality.[10] Timely diagnosis and management of ART failure and drug resistance in this patient population has potential to contribute to the UNAIDS 95-95-95 targets set for 2030, as well as improving individual patient outcomes.[1] Currently, few data exist describing HIV drug resistance in patients established on ART but admitted to hospital.

To describe the prevalence of virological failure and HIV drug resistance, and their impact on early mortality in HIV-positive patients admitted to hospital in high-HIV burden settings, we undertook an observational cohort study nested within a large tuberculosis screening trial in SSA.

Methods

Study setting, design and procedures

This cohort study was nested within the Rapid Urine-based Screening for Tuberculosis (TB) to Reduce AIDS Related Mortality in Hospitalised Patients in Africa (STAMP) trial, which recruited unselected (i.e. irrespective of clinical presentation), adult (aged ≥18 years) HIV-1 positive patients at admission to medical wards.[4,11] Enrolled patients were randomised to TB screening using sputum testing alone, or sputum and urine testing. TB screening results were provided to clinical teams, but

with all patient management provided by hospital clinicians without input from study staff. All patients were managed as per national HIV guidelines (appendix, page 2).

Exclusion criteria were: recent TB treatment (last 12 months) or TB preventative therapy (last 6 months), or being unable or unwilling to provide informed consent. The study team recorded clinical data at admission and during hospitalisation on standardised case report forms based on patient interview, medical records and clinical review. Patients discharged alive were followed-up at 56-days. Vital status was established by home visit, telephone or through next of kin for those not attending follow-up.

Patients were included in this study if they were enrolled at the Malawi site (Zomba Central hospital, see appendix, page 3), and were taking ART for at least 6 months at admission. A random sample of 80 patients not currently taking ART were also included to provide data on pre-treatment HIV drug resistance. Data on ART status at admission were collected by patient interview and confirmed by reviewing hand-held outpatient notes ("health passport") and/or ART prescription. At enrolment, patients underwent venepuncture for CD4 cell count, haemoglobin, and plasma which was stored at -80˚C.

Management of HIV was as per national HIV guidelines.[12] First-line ART was tenofovir, lamivudine and efavirenz since 2011, and viral load monitoring was recommended routinely at 6 months, 2 years and then 2 yearly after commencing ART, and if virological failure is clinically suspected (appendix, page 3). Viral load testing is not routinely done for hospital inpatients, but is mandatory prior to switching to second-line ART.

All patients provided informed written consent for participation and sample storage. Retrospective HIV viral load and drug resistance testing was approved by the research ethics committee of the London School of Hygiene and Tropical Medicine, and the University of Malawi College of Medicine Research and Ethics Committee (COMREC). This study confirms to STROBE guidelines for observational studies (appendix, page 8).

Laboratory methods and HIV genotyping

HIV plasma viral load measurements from frozen plasma used Abbott RealTime HIV-1 (m2000sp) (Abbott Molecular Inc, IL USA) were undertaken in Malawi. For enrolled patients with virological failure, defined as a plasma viral load ≥1000 copies per mL, HIV-1 genotyping by ultra-deep sequencing was performed to detect drug resistance mutations (DRMs) in the UK.

Nucleic acids were extracted from 230 µL of plasma using DSP Virus/Pathogen kit on the QIAsymphony platform and amplified using in-house HIV primer sets (gag-pol codons 691-3582, pol-

int gag-pol codons 2696-5527, int-env (g120) gag-pol codons 5518-7374). Library preparations were generated using the Nextera®XT DNA Sample Preparation Kit and sequenced on Illumina MiSeq platform (Illumina Inc). Bioinformatic analysis was done using the "de novo" Iterative Virus Assembler (IVA, http://sanger-pathogens.github.io/iva). Following sequencing, samples were aligned using the MAFFT program (version 7, https://mafft.cbrc.jp/alignment/software/).

HIV-1 subtype and drug resistance mutations were analysed using the Stanford HIVdb Program (version 2.3.0 https://hivdb.stanford.edu/).[13] Drug resistance mutations were only considered if present in ≥20% of viral population. The level of drug resistance was determined by adding penalty scores for each drug resistance mutation according to the Stanford HIVdb algorithm (version 8.8), with the level (1 to 5) being calculated based on the total score.[14] Drug resistance was defined as level 4 (intermediate) or level 5 (high-level) resistant. Multidrug resistance (MDR) was defined as resistance to two or more first-line drugs from the first line ART regimen.[15]

Statistical analysis

Categorical data were compared using chi-square tests, continuous data using t-tests or Wilcoxon rank sum dependent on distribution. Mortality risk was calculated 56 days from enrolment patients were censored at death, 56-days or at last contact if lost to follow-up. Cox proportional hazards models were used to assess associations with mortality, p-values were calculated using likelihood ratio tests. The modelling strategy addressed the causal association between HIV multidrug resistance and mortality, and excluding factors on the causal pathway (most notably, CD4 cell count, clinical signs of advanced HIV and poor functional and nutritional status). All models were adjusted for STAMP trial arm.

In the Cox regression analysis, linear association and departures from linearity of continuous variables with log(mortality rate) was assessed using the likelihood ratio test. For the mortality regression analysis, all patients with viral load <1000 copies per mL were assumed to have no drug resistance, and patients with virological failure but missing drug resistance data were excluded. A sensitivity analysis was done only including patients on ART for >12 months. Results are reported as hazard ratios (HR) with 95% confidence intervals (CIs) and Kaplan-Meier curves. Analyses used Stata version 14 (College Station, Texas, USA). For statistical analysis plan see appendix (page 13).

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Of 1316 HIV-positive patients enrolled in the STAMP trial at the Malawi site between October 2015 and September 2017, 84·2% (n=1108) knew their HIV status, of whom 92·1% (1021) were taking ART, with 814 (79·7%) on ART for at least 6 months at hospital admission. 28 patients had missing HIV viral load measurements, leaving 786 included in the analysis (figure 1). Mean age was 41·5 years, 32·8% were male, 98·0% (770/786) were on first-line ART and the median time on ART was 4·9 years (IQR 2·2 to 8·1 years), with 77·1% having advanced HIV as defined by CD4 count below 200 cells per µL or WHO stage 3 or 4 illness (table 1).

59·5% (468/786) of patients had undetectable HIV-1 viral loads (<50 copies per mL) and 32·1% (252/786) had virological failure (viral load ≥1000 copies per mL, median viral load 125,603 copies per mL), and a further 8·4% (66/786) having low-level viraemia (between 50 and 999 copies per mL). Patients with virological failure were younger, more likely than other patients to be male, to have lower body mass index (BMI), Karnofsky scores and CD4 cell counts (60 compared to 383 cells per µL), with 93·6% having advanced HIV at admission. They were also more likely to receive antimicrobial and TB treatment as inpatients, and had a longer median length of hospitalisation (17 compared to 14 days, table 1). Of the 16 patients on second-line ART, 6 (37·5%) had virological failure.

Of 252 samples from patients with virological failure, 237 (94·0%) were successfully sequenced (237, 233 and 225 had reverse transcriptase, protease and integrase genes sequenced, respectively) with all being HIV subtype C. 93.2% (221/237) of patients had DRMs to first or second-line drugs.

The most common relevant NRTI DRMs were Met184Val (75·1%, n=178) and Lys65Arg/Asn (40·9%, n=97) conferring drug resistance to lamivudine and tenofovir respectively (table 2, figure 2).[16] 84 (35·4%) patients had at least one thymidine analogue mutation (TAM), and 55 (23.2%) had at least 3 TAMs. Resistance to lamivudine was seen in 82·3% (n=195), and tenofovir disoproxil in 54·0% (n=128). Resistance to other NRTIs was also common, with 76·4% (n=181) having drug resistance to abacavir and 25·3% (n=60) to zidovudine. In 16·9% (40/237) of patients, HIV was susceptible to all NRTIs, and in 67·9%, HIV was resistant to 3 or all 4 available NRTI drugs.

NNRTI resistance was almost universal, with 92·4% (n=219) of samples resistant to efavirenz, and 92·8% (n=220) resistant to nevirapine. The most common NNRTI mutations were Lys103Asn/Ser/His (39.7%, n=94), Tyr181Cys/Ile/Glu (36·7%, n=87) and Gly190Ala/Ser/Glu (41·4%, n=98). Resistance to newer NNRTI drugs was also common, consistent with anticipated cross-resistance, with 66·7% (n=158) resistant to rilpivirine, and 61·6% (n=146) resistant to etravirine.

PI and integrase inhibitor (INI) resistance were rare, with only 1/233 (0·4%) patient having a major PI mutation (Val82Ala), and two patients (0.9%) having accessory PI mutations (Gln58Glu). 2/225 (0·9%) patients also had major INI mutations (one Gln148His, and one Thr66Ser), and 8/225 (3·6%) had accessory INI mutations (six Glu157Gln and two Gln95Lys).

Overall, only 7·6% (n=18) of patients had no detectable resistance to any first-line ART drugs, with 53·6% (n=127) having resistance to all first-line drugs, and 82·7% (n=196) to at least two drugs (table 2, appendix page 5). Assuming patients with HIV viral load <1000 copies per mL had no drug resistance, the prevalence of resistance to at least two first-line ART drugs (multidrug resistance) was 25·4% (196/771) for patients hospitalised after taking ART for six months or longer. 8/15 patients with viral loads between 400 and 999 copies per mL also had HIV-1 genotyping, of which 7/8 (87·5%) had MDR.

56-day mortality was 19·8% (156/786, 95% CI 17·2 – 22·8%), with over half (83/156, 53·2%) of deaths occurring after discharge from hospital. Mortality was greater in those with virological failure compared to those without (mortality 24·6% and 17·6% respectively, unadjusted HR 1·44 [95% CI 1·04-1·98] p=0.028). This difference mainly reflects deaths after discharge (17.1% [95% CI 12·7-22·6%] versus 9·1% [95% CI 6·8-12·0%], p=0·0020) with inpatient mortality similar for those with and without virological failure (9·1% and 9·4% respectively).

Among 237 patients with virological failure and available HIV drug resistance genotypes, unadjusted mortality increased with increasing drug resistance. 2-month mortality was 5.6% in patients with no drug resistance, 13.0% if resistant to one drug, and 28.1% with resistance to two or more first-line ART drugs (multidrug resistance, MDR, p=0.041, appendix page 6).

In analyses adjusted by STAMP trial arm only, age, sex, time on ART, advanced HIV, BMI, Karnofsky score, CD4 count, haemoglobin, WHO danger signs, TB treatment and virological failure were all strongly associated with increased mortality (table 3). HIV multidrug resistance was associated with increased mortality (HR 1·7, 95% CI 1·2-2·3, p=0·0024), and remained so after adjustment for age, sex, time on ART, TB treatment and trial randomisation arm (adjusted HR 1·7, 95% CI 1·2-2·4, p=0·0042) (table 3, figure 3). There were no significant interactions between variables in the final model. Sensitivity analyses only including patients currently taking ART for 12 months or longer yielded similar results (n=724, adjusted HR 1·9, 95%CI 1·3-2·7, p=0·0011).

In exploratory analyses, adjusting for CD4 cell count, clinical signs of advanced HIV and poor functional or nutritional status mitigated the association of HIV MDR and mortality, supporting their position on the causal pathway between multidrug resultant HIV and death, and their exclusion from the multivariable causal model.

79 samples from patients not taking ART at admission were successfully sequenced, among whom 60 patients were ART naïve, and 19 had previously taken ART. Baseline characteristics differed (appendix, page 7) with patients never taking ART more likely to be male, with less advanced HIV, higher BMI, higher Karnofsky score and higher CD4 counts. HIV viral load was also higher in ART naïve patients (median 603,000 copies per mL, IQR 66,600 – 1,300,000 copies per mL). HIV drug resistance was uncommon among ART naïve patients, with no NRTI DRMs (table 2) but 11·7% (7/60) with NNRTI resistance to efavirenz and nevirapine. Among 19 patients with previous ART exposure, two (10·5%) had NRTI resistance to lamivudine, tenofovir and abacavir, and 8 (42·1%) had resistance to efavirenz. No major mutations to PIs or INIs were detected.

Discussion

Our findings show that, in Malawi, the vast majority of HIV positive inpatients knew their HIV status and were taking ART, in contrast to previously reported hospital cohorts.[2] However, virological failure was common (32.1% of patients taking ART for 6 months at admission) and HIV drug resistance was almost universal amongst patients with virological failure (82·7% resistant to two or more ART drugs). Importantly, our data show the impact of HIV drug resistance with increased short-term mortality risk. Pre-treatment drug resistance was restricted to NNRTIs, consistent with findings from recent African community-based surveys.[7,9]

This is the first study to report virological failure and HIV drug resistance in unselected HIV positive patients admitted to hospital in SSA, and to report mortality outcomes. Hospital inpatients are an important source of information on the major causes of severe illness and death in key subpopulations such as people living with HIV. Consistent with regional HIV care cascade data, our data show undiagnosed and untreated HIV was a less common cause of severe illness than previously reported, given the high proportion of patients already taking ART at admission.

Our finding that 19·1% of all admissions among PLHIV (24·7% of patients taking ART for any duration) had virological failure highlights the growing importance of drug resistance and system weaknesses that limit diagnosis and management of virological failure before the onset of critical illness. Other African data also show widespread ART failure among inpatients, and to a lesser extent outpatients.[10,17] Our data showing that drug resistance as an important cause of admissions and deaths are, therefore, likely to be regionally generalisable.

In our study, virological failure was synonymous with HIV drug resistance. NNRTI resistance occurred in 92.4%, unsurprising given widespread use and low barrier to resistance, but resistance was more common than in outpatient studies.[17] We also saw high rates of resistance to newer NNRTIs (etravirine and rilpivirine), likely due to prior exposure to nevirapine.[18] NRTI resistance was also

common, 82.3% to lamivudine and 54.0% to tenofovir (widely used in SSA), with resistance to other NRTIs also widespread. The high prevalence of TAMs (35% had one or more, 23% had three or more) likely reflects the prior stavudine exposure. The prevalence of Lys74Ile as a compensatory mutation for Met184Val/Ile also suggests that these individuals had been failing first line ART for a significant period of time.[19] Although PIs and INIs are included in alternative ART regimens,[20] the lack of major resistance to these drug classes reflects little drug exposure and high barriers to resistance. The low prevalence of pre-treatment HIV drug resistance implies acquisition of drug resistance during treatment, most likely driven by sub-optimal adherence. We also demonstrated multidrug resistance in patients with low-level viraemia, the significance of which remains unclear.

Mortality in this cohort was high, 20% by 2-months. We found markers of advanced HIV disease were associated with mortality. HIV drug resistance was also associated with mortality, showing a dose-response relationship, and multidrug resistance was independently associated with mortality. Viral load testing is rarely done for inpatients, and centralised testing programs with long turnaround times and lacking electronic laboratory management systems mean patients will have either died or been discharged once results are available.[21] Therefore, virological failure in this study would have gone undetected and untreated.

These findings suggest interventions aimed at preventing and diagnosing virological failure and drug resistance could reduce morbidity and mortality in patients taking ART. Prior to hospital admission, patients were actively attending ART clinics that failed to identify and address their ART failure and advanced HIV. Whilst the public health approach to ART has led to declines in HIV incidence and mortality, patients with advanced disease (despite engagement with clinics) may well benefit from differentiated care with focused approach to patients with high viral loads, including more frequent viral load testing, review and monitoring.[22,23] This may be challenging with increased task-shifting and increasingly complex ART regimens and interactions, although clinical decision tools may help identify patients at high risk of poor outcomes.[24]

Currently, HIV drug resistance testing has not been prioritised by HIV programmes in high burden settings in SSA. Point-of-care technologies to detect important drug resistance mutations are in the pipeline, and their use to guide ART choice led to improved outcomes among patients with pre-treatment drug resistance prior to initiation of first line ART.[25,26] Our findings should encourage investment in resistance testing for patients with repeat high viral loads.

In hospital, patients taking ART who have signs of advanced HIV would benefit from rapid, near-patient testing for virological failure, for example using the Xpert HIV-1 Viral Load assay (Cepheid, Sunnyvale, CA, USA). Its use was recently shown to increase viral suppression and retention in care in South Africa.[27] Patients identified as failing ART will need switching to alternative regimens

given high prevalence of drug resistance with adherence support. Screening and/or empirical treatment for opportunistic infections is also recommended by WHO advanced HIV guidelines.[5] Post-hospital care, especially for patients failing ART, should be improved. Systems to ensure post-discharge follow-up and monitoring of patients with ART failure may help reduce the high mortality seen in the weeks following discharge.

Malawi and WHO guidelines for ART introduced dolutegravir-based regimens (with tenofovir and lamivudine NRTI backbone) as the preferred first line ART from 2019.[20,28] This has important implications for our findings, as dolutegravir will likely overcome the high levels of NNRTI resistance, has high barriers to resistance and can be co-administered with TB treatment.[29,30] However, the high prevalence of NRTI resistance (68% resistant to three or more NRTIs) suggest significant numbers could be on functional dolutegravir monotherapy. Data from the EARNEST trial demonstrated that even in the presence of predicted NRTI resistance, virological suppression with PI and NRTI based-regimens was good (89%).[31] However, these patients were not acutely unwell, and recent data suggest that dolutegravir may be less potent than previously expected in patients with high viral loads,[32] and have a lower barrier to resistance as compared with ritonavir-boosted PIs when used as monotherapy.[33] The real-world outcomes from dolutegravir-based ART and the impact of MDR HIV therefore remains to be established.

The strengths of our study are that it is a large cohort nested in a clinical trial, and unselected HIV-positive patients were enrolled, reducing bias. Furthermore, the data reflect routine clinical care in hospitalised patients. There are also some limitations. It is a single-centre study, although results are likely to be generalisable to other SSA settings. There is a small number missing data on HIV viral load and/or HIV drug mutations. The exclusion of patients not consenting may have underestimated virological failure and MDR, as these are likely to be sicker patients. We assumed that patients with viral loads <1000 copies per mL had no drug resistance, but the prevalence and implications of resistance in this group is not clear. We did not have detailed adherence information for patients. However, patients all self-reported taking ART and had evidence of attending ART clinics and ART being dispensed. Furthermore, high prevalence of drug resistance also supports that patients were taking drugs, as reversion to wildtype is common without drug pressure.

In conclusion, we have found virological failure and HIV drug resistance to be extremely common in HIV-positive inpatients, and drug resistance was associated with increased mortality. Patients already established on ART with advanced HIV disease need screening for failure during hospital admission, ideally using rapid assays. Those identified as failing ART would likely benefit from switching to alternative ART (integrase or PI-based given NNRTI and NRTI resistance). Interventions targeting ART clinic and post-hospital care are also needed. Our findings also support the development of low-cost and rapid assays to detect HIV drug resistance.

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Data Sharing:

Data will be publicly available through London School of Hygiene & Tropical Medicine Data Compass repository (DOI to be provided prior to publication). HIV-1 genotypes are in the process of being uploaded to GenBank (accession number(s) to be provided prior to publication).

Author Contributions:

AGW, KF, JvO and ELC contributed to study conception. AGW, KF, JvO, MA, EC, EN, HCM, ELC and RKG contributed to study design. KF and ELC obtained the funding. AGW, JvO, MA, EC and JH contributed to data collection. AGW, KF, DG and MB contributed to data analysis. AGW, KF, JvO, JH, EN, HCM, ELC and RKG contributed to data interpretation. AGW, KF, ELC and RKG wrote the first draft. All authors read and approved the final manuscript.

Declaration of Interests

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Table 1: Patient characteristics at baseline and outcomes, stratified by virological failure

		Overall n=786	HIV viral load <1000 copies per mL n=534	HIV viral load ≥1000 copies per mL n=252	p-value
Baseline characteristics					
Age (years)	mean (SD)	41.5 (11.4)	43.1 (11.8)	38.2 (9.8)	<0.0001
Sex	Male Female	258 (32.8%) 528 (67.2%)	161 (30.1%) 373 (69.9%)	97 (38.5%) 155 (61.5%)	0.020
Time on ART	median (IQR)	4.7 (2.0, 8.1)	4.6 (1.9, 8.1)	5.0 (2.5, 8.1)	0.16
ART regimen	1st Line 2nd line	770 (98.0%) 16 (2.0%)	524 (98.1%) 10 (1.9%)	246 (97.6%) 6 (2.4%)	0.64
Advanced HIV	Yes	606 (77.1%)	370 (69.3%)	236 (93.7%)	<0.0001
WHO TB 4 symptom screen	Yes	606 (77.1%)	467 (87.5%)	238 (94.4%)	0.0026
Body mass index (kg/m^2, BMI)	mean (SD)	19.8 (4.2)	20.5 (4.2)	18.3 (3.6)	<0.0001
WHO danger sign	Yes	191 (24.3%)	120 (22.5%)	71 (28.2%)	0.082
Karnofsky score	median (IQR)	60 (50, 70)	60 (50, 70)	50 (50, 60)	<0.0001
CD4 count (cells/µL)[a]	median (IQR)	277 (97, 496)	383 (234, 562)	60 (17, 156)	<0.0001
HIV viral load (copies per mL)	median (IQR)	0 (0, 14782)	-	-	-
Haemoglobin (g/l)	mean (SD)	102.1 (31.0)	107.4 (31.5)	90.7 (26.8)	<0.0001
Treatments and outcomes					
STAMP trial randomisation arm	Standard of Care	402 (51.1%)	267 (50%)	135 (53.6%)	0.35
	Intervention	384 (48.9%)	267 (50%)	117 (46.4%)	
Received any anti-microbial treatment	Yes	695 (88.4%)	457 (85.6%)	238 (94.4%)	0.0003
Received TB treatment	Yes	109 (13.9%)	66 (12.4%)	43 (17.1%)	0.075
Length of stay (days)	median (IQR)	15.0 (8.0, 21.0)	14.0 (5.0, 21.0)	17.0 (14.0, 21.0)	0.018
Visited ART clinic after discharge	Yes	475 (66.9%)	312 (64.7%)	163 (71.5%)	0.074
Mortality at 56 days	Overall	156 (19.8%)	94 (17.6%)	62 (24.6%)	0.022
	During hospital admission	73 (9.3%)	50 (9.4%)	23 (9.1%)	0.92
	After discharge from hospital	83 (11.7%)	44 (9.1%)	39 (17.1%)	0.0019

P-values compare HIV viral <1000 copies per mL and ≥1000 copies per mL, calculated using chi-squared for proportions, t-tests for means and Wilcoxson rank sum for medians. [a] 2 patients missing CD4 count results. ART Antiretroviral therapy; WHO World Health Organization; SD standard deviation; IQR

interquartile range. Advanced HIV defined as CD4 count <200 cells/µL or stage 3 or 4 illness. WHO 4 symptom TB screen is defined as ≥1 of current cough, fever, weight loss or night sweats WHO Danger sign is as ≥1 of respiratory rate >30 per minute, temperature >39°C, heart rate >120 beats per minute and unable to walk unaided.

Table 2: HIV drug resistance by ART status at admission

	Taking ART ≥6 months with HIV viral load ≥1000 copies per mL (%) n=237	ART naïve (%) n=60	ART prior experience (%) n=19
Resistance to NRTIs			
lamivudine	195 (82.3)	0	2 (10.5)
tenofovir	128 (54.0)	0	2 (10.5)
abacavir	181 (76.4)	0	2 (10.5)
zidovudine	60 (25.3)	0	0
stavudine	167 (70.5)	0	2 (10.5)
didanosine	169 (71.3)	0	2 (10.5)
Resistance to NNRTIs			
efavirenz	219 (92.4)	7 (11.7)	8 (42.1)
nevirapine	220 (92.8)	7 (11.7)	9 (47.4)
rilpivirine	158 (66.7)	2 (3.3)	5 (26.3)
etravirine	146 (61.6)	2 (3.3)	3 (15.8)
Resistance to PIs[a]			
ritonavir-boosted lopinavir	1 (0.4)	0	0
ritonavir-boosted atazanavir	0	0	0
ritonavir-boosted darunavir	0	0	0
Resistance to INIs[b]			
raltegravir	1 (0.4)	0	0
dolutegravir	0	0	0
Resistance to first-line ART			
Susceptible to all drugs	18 (7.6)	53 (88.3)	11 (57.9)
Resistance to 1 drug	23 (9.7)	7 (11.7)	6 (31.6)
Resistance to 2 drugs	69 (29.1)	0	0
Resistance to 3 drugs	127 (53.6)	0	2 (10.5)
Resistance to ≥2 drugs	196 (82.7)	0	2 (10.5)
NRTI mutation			
Lys65Arg/Asn	97 (40.9)	0	1 (5.3)
Met184Val	178 (75.1)	0	2 (10.5)
Leu74Ile	17 (7.2)	0	0
Leu74Val	3 (1.3)	0	0
Thymidine analogue mutation (TAM)			
Met41Leu	37 (15.6)	0	0
Asp67Asn	31 (13.1)	0	0
Lys70Arg	21 (8.9)	0	0
Leu210Trp	6 (2.5)	0	0
Thr215Phe/Tyr	42 (17.7)	0	0
Lys219Gln/Glu	37 (15.6)	0	0
≥1 TAMs	84 (35.4)	0	0
≥3 TAMs	55 (23.2)	0	0
NNRTI mutation			
Leu100	23 (9.7)	0	1 (5.3)
Lys103Asn/Ser/His	94 (39.7)	4 (6.7)	5 (26.3)

Tyr181Cys/Ile/Val	87 (36.7)	1 (1.7)	2 (10.5)
Tyr188Leu/Cys/His	19 (8.0)	0	1 (5.3)
Gly190Ala/Ser/Glu	98 (41.4)	0	2 (10.5)
Met230Leu/Ile	4 (1.7)	0	0
PI and INI mutation[ab]			
Val82Ala	1 (0.4)	0	0
Gln148Gln/His	1 (0.4)	0	0
Thr66Thr/Ser	1 (0.4)	0	0

Data represent individual participant numbers, brackets denote %. ART antiretroviral therapy; NRTI nucleoside reverse transcription inhibitor; NNRRTI non-nucleoside reverse transcription inhibitor; PI protease inhibitor; INI integrase inhibitor; TAM Thymidine analogue mutation. [a]missing data for 4 patients whose protease gene was not successfully sequenced. [b]missing data for 12 patients whose integrase gene was not successfully sequenced.

Table 3: Number of patients who survived and died by 56-days and univariable and multivariable Cox regression analysis of mortality

		Died[a] n=156	Univariable HR for mortality (95% CI)	p-value[g]	Multivariable HR for mortality (95% CI)	p-value[g]
Age (years)[b]	mean (SD)	43.4 (12.8)	1.02 (1 - 1.03)	0.022	1.02 (1.01 - 1.04)	0.0049
Sex	Male	83 (32.2)	1		1	
	Female	73 (13.8)	0.39 (0.28 - 0.53)	<0.0001	0.49 (0.35 - 0.67)	<0.0001
Time on ART	median (IQR)	3.9 (1.6, 7.0)	0.95 (0.91 - 0.99)	0.023	0.94 (0.90 - 0.99)	0.016
ART regimen	1st Line	152 (19.7)	1			
	2nd line	4 (25)	1.32 (0.49 - 3.56)	0.60		
Advanced HIV	No	17 (9.4)	1			
	Yes	139 (22.9)	2.61 (1.58 - 4.32)	<0.0001		
WHO 4 symptom TB screen	No	13 (16)	1			
	Yes	143 (20.3)	1.28 (0.73 - 2.26)	0.37		
Body mass index	mean (SD)	18.3 (3.6)	0.88 (0.84 - 0.92)	<0.0001		
WHO danger sign	No	93 (15.6)	1			
	Yes	63 (33)	2.37 (1.72 - 3.26)	<0.0001		
Karnofsky score	median (IQR)	50 (40, 50)	0.94 (0.93 - 0.96)	<0.0001		
CD4 count[c]	median (IQR)	135 (42, 299)	0.89 (0.86 – 0.93)[e]	<0.0001		
HIV viral load (copies per mL)	<1000	97 (17.6)	1	0.028		
	≥1000	59 (24.9)	1.44 (1.04 - 1.98)			
Haemoglobin (g/l)	mean (SD)	86.3 (29.3)	0.84 (0.80 - 0.88)[f]	<0.0001		
Received any anti-microbial treatment	No	17 (18.7)	1			
	Yes	139 (20)	1.1 (0.67 - 1.83)	0.69		
TB treatment	No	116 (17.1)	1		1	
	Yes	40 (36.7)	2.36 (1.65 - 3.39)	<0.0001	2.12 (1.47 - 3.06)	0.0002
Multidrug resistant HIV[d]	No	100 (17.2)	1		1	
	Yes	56 (27.6)	1.69 (1.21 - 2.34)	0.0024	1.68 (1.19 - 2.37)	0.0042

[a] data represent individual participant numbers (%), mean (standard deviation) or median (interquartile range). [b] unadjusted HR for a 10 year increase in age is 1.17 (95% CI 1.02 – 1.34), adjusted HR for a 10 year increase in age is 1.24 (95% CI 1.07 – 1.43). [c] CD4 count data missing for 2 patients. [d] defined as resistance to two or more first-line ART drugs, data missing for 15 patients without HIV drug resistance data, patients with suppressed virus (<100 copies per mL) were assumed to have no drug resistance. [e] HR is a 50 cells per mL increase in CD4 count. [e] HR is for a 10 g/l increase in haemoglobin. For all other continuous variables HR represent a one unit increase. There was no evidence for departures from linearity for any continuous variables. [g] p-values calculated using the likelihood ratio test from the Cox

proportional hazards model. All models adjusted for STAMP trial randomisation arm. No interactions in adjusted model. ART antiretroviral therapy; TB tuberculosis; WHO World Health Organization; HR hazard ratio. Advanced HIV defined as CD4 count <200 cells/µL or stage 3 or 4 illness. WHO 4 symptom TB screen is defined as ≥1 of current cough, fever, weight loss or night sweats WHO Danger sign is as ≥1 of respiratory rate >30 per minute, temperature >39°C, heart rate >120 beats per minute and unable to walk unaided.

1316 patients recruited from the Malawi site of the STAMP trial

208 new HIV diagnosis

1108 (84.2%) known HIV status prior to admission

57 ART naïve
30 Stopped ART

1021 (92.1%) Currently taking ART

207 ART <6 months or missing time on ART
28 missing HIV viral load

786 included in the study

Viral Load <1000 copies/ml: 534 (67.9%)

Viral Load ≥1000 copies/ml: 252 (32.1%)

Figure 1: Study flow diagram for inclusion into this observational cohort study

ART is antiretroviral therapy

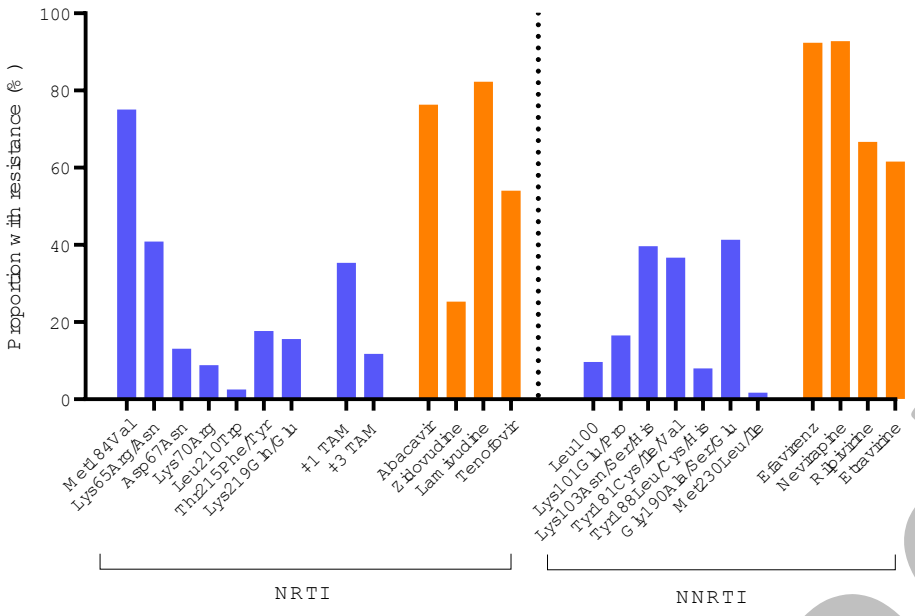

Figure 2: Proportion of patients with virological failure who have HIV drug resistance mutations and intermediate or high level resistance to first-line ART drugs

N=237. ART antiretroviral therapy; NRTI nucleoside reverse transcriptase inhibitor; NNRTI non-nucleoside reverse transcriptase inhibitor; TAM Thymidine analogue mutation.

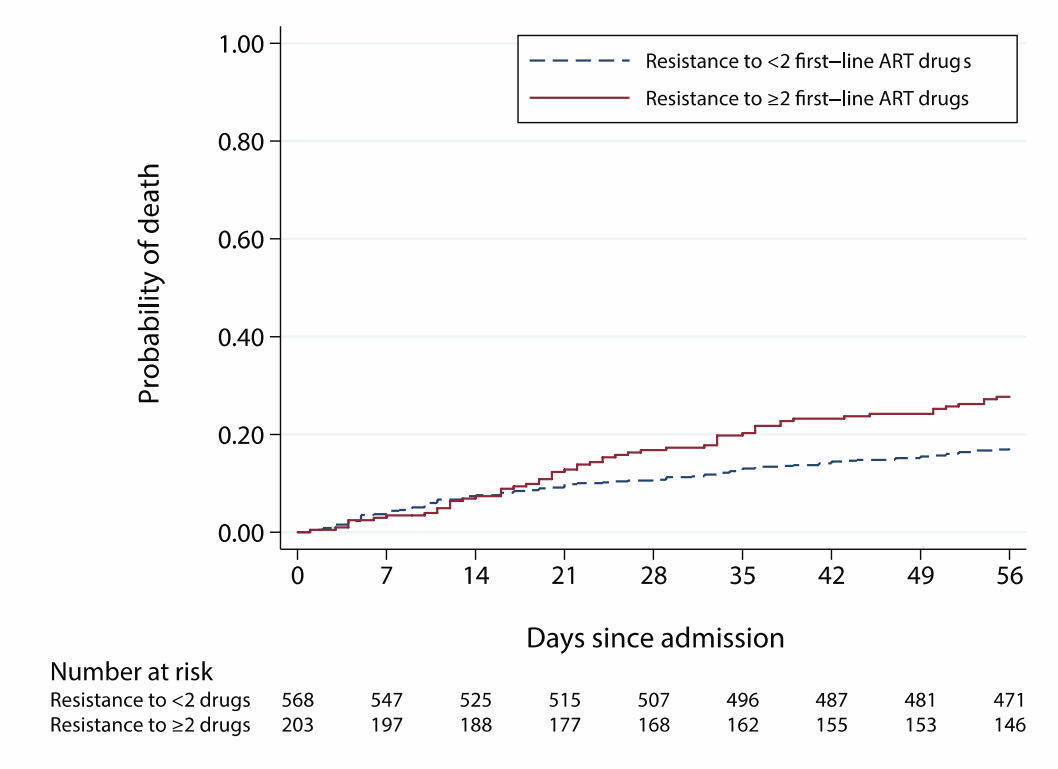

Figure 3: Kaplan Meier curve showing probability of death stratified by drug resistance

Kaplan Meier curve shows time to death stratified by resistance to <2 first-line ART drugs, or resistance to or ≥2 first-line ART drugs (unadjusted HR 1.7, 95% CI 1.2-2.3, p=0.0024, adjusted HR 1.7, 95% CI 1.2-2.4, p=0.0042; log-rank p=0.0002). Patients with viral loads <1000 copies per mL were assumed to have no resistance, 15 patients without HIV drug resistance data are excluded (n=771). ART antiretroviral therapy.