HIV drug resistance and third line treatment outcomes in patients failing: Protease Inhibitor based second-line antiretroviral therapy in Zimbabwe

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Doctorate Thesis

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2. *Chimbetete C*, Shamu T, Keiser O. Zimbabwe's National Third-Line Antiretroviral Therapy Program: Cohort Description and Treatment Outcomes (Accepted for publication in Plos One. Still in production)
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3. *Oral Presentation:* A case report of untreatable HIV infection in Harare, Zimbabwe. 27th International Workshop on HIV drug resistance and treatment strategies. South Africa 2018

Publications not related to thesis but done and supervised by PHD supervisor

1. *Chimbetete C*, Mugglin C, Shamu T, Kalesan B, Bertisch B, Egger M, et al. New-onset type 2 diabetes mellitus among patients receiving HIV care at Newlands Clinic, Harare, Zimbabwe: retrospective cohort analysis. Trop Med Int Heal. 2017;22. doi:10.1111/tmi.12896
2. *Chimbetete C,* Roelens M, Shamu T, Bote S, Mudzviti T, Keiser O. Mortality trends and causes of death among HIV infected patients at Newlands Clinic in Harare, Zimbabwe (Under Review in Plos One Journal)

Abstract

Introduction

Zimbabwe is among the countries in the world that are most affected by human immunodeficiency virus (HIV) infection and has a large HIV treatment programme with over a million people receiving antiretroviral therapy (ART). HIV treatment is provided using the public health approach with guidance from the World Health Organization (WHO). The public health approach utilizes standard first, second and third-line ART for the management of HIV infection. Third-line ART was introduced in Zimbabwe in 2015 through four designated national third-line treatment centers. All the four centers are designated specialist HIV treatment clinics with access to laboratory and radiology support. Patients failing second-line ART are transferred to the nearest third-line center by their treating physicians. These patients are supposed to receive at least three months of enhanced adherence support before being offered HIV drug resistance (HIVDR) testing. National standard operating procedures recommend that patients must have confirmed good adherence to second-line ART to become eligible for HIVDR testing. The Stanford HIV Drug Resistance Database is used to assess resistance to ART. HIVDR testing is not available in the public sector and hence patients need to access testing in the private sector at a prohibitive cost. Second-line treatment failure is confirmed by the presence of any level of resistance to atazanavir and lopinavir. Third-line ART for adults and children is composed of boosted darunavir, dolutegravir / raltegravir (raltegravir was used between 2015 and 2017 before dolutegravir availability) and sometimes two additional nucleoside or nucleotide reverse transcriptase inhibitors (N(t)RTIs) (based on the HIVDR test results). We set out to assess the distribution of HIV drug resistance mutations and their risk factors in patients failing 2nd line ART and to determine treatment outcomes among patients receiving 3rd line ART in Zimbabwe.

Methods

This work was done in collaboration with the Zimbabwe Ministry of Health through the AIDS and TB program. Four national third-line centers were established to provide treatment for patients failing second-line ART. A national standard operating procedure for the management of patients failing second-line ART was developed. At one of the treatment centers, Newlands Clinic, patients failing second-line ART were enrolled into an adherence support group which met once a week (2.5 hours) for a minimum of six weeks. HIVDR testing was done for patients suspected of second-line failure with a confirmed viral load >1000 copies/mL after 6 weeks of enhanced adherence support, and had good adherence, as per national guidelines and sequences were analyzed using the Stanford University HIV Drug Resistance Database's HIVdb program, version 8.3. Third-line treatment outcomes were assessed using viral load measurements, deaths and loss to follow up.

Results

In our first publication: A total of 186 participants received adherence support for second-line failure, 61 achieved post adherence support viral loads of less than 1000 copies/mL, 3 were lost to follow-up, 1 was transferred out, and 35 did not meet clinical criteria for genotyping due to confirmed poor adherence. Of the 86 patients who had HIVDR testing, only 50 (58%) had major protease inhibitor mutations. Among the 36 patients who received third-line ART, early treatment outcomes were excellent with 29 (81%) achieving week 24 viral loads of <50 copies/ml.

In our second publication: A total of 209 patients had ever received third-line ART: 124 at Newlands Clinic (NC) and 85 from the three government clinics. HIV genotype results were available for 89 (72%) patients at NC and fourteen (16.5%) patients in the government clinics. Median duration of third line ART (years) in the government clinics was 2.3 (IQR:0.6-3.4), 1.3 (IQR: 0.7-1.7) and 1 (0.6-1.9). Of the 67 patients who received third line ART in the government

clinics for at least six months, 53 (79%) had most recent viral load (VL) < 1000 copies/ml. Data on other treatment outcomes from government clinics were incomplete. From NC: a total of 109 (88%) patients were still in care, 13 (10.5%) had died and 2 (1.5%) were transferred. Median duration of third-line ART was 1.4 years (IQR: 0.6-2.8). Among the 111 NC patients who had received third-line ART for at least 6 months, 83 (75%) had a VL <50 copies/ml and 106 (95.5%) had a VL <1000 copies/ml.

Conclusion

Our findings demonstrate that poor adherence is the major cause of second-line ART failure. Furthermore, with comprehensive care, patients failing second-line ART can achieve high rates of virological suppression on third-line ART regimens. Access to HIV genotyping in Zimbabwe is low and may be a barrier to effective diagnosis of second-line ART failure and inappropriate switches to third-line ART.

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Résumé

Introduction

Le Zimbabwe est l'un des pays au monde le plus touché par le virus de l'immunodéficience humaine (VIH) et celui-ci dispose d'un vaste programme de traitement du VIH avec plus d'un million de personnes recevant un traitement antirétroviral (TARV). Ce traitement est dispensé selon les schémas thérapeutiques de santé publique et les recommandations de l'Organisation mondiale de la santé (OMS). Ces schémas utilisent les TARV standards de première, deuxième et troisième intention pour les traitements de l'infection par le VIH. Le TARV de troisième intention a été introduit au Zimbabwe en 2015 dans quatre cliniques spécialisées dans le traitement du VIH, désignées comme centres nationaux, ayant chacune leur propre laboratoire et service de radiologie. Les patients dont le TARV de deuxième intention échoue sont transférés dans le centre de troisième intention le plus proche par leur médecin traitant. Ces patients sont censés recevoir au moins trois mois de soutien renforcé à l'adhérence thérapeutique avant de se voir proposer un test de résistance aux médicaments anti-VIH (RMHIV). Les procédures opératoires nationales standards recommandent que les patients doivent avoir une bonne adhérence confirmée du TARV de deuxième intention afin de devenir éligibles au test de RMVIH. La base de données sur la résistance aux médicaments contre le VIH de Stanford est utilisée pour évaluer la résistance aux TARV. Le test de RMVIH n'est pas disponible dans le secteur public et, par conséquent, les patients doivent se faire tester dans le secteur privé à un coût prohibitif. L'échec du traitement de deuxième intention est confirmé par la présence de résistance à l'atazanavir et au lopinavir (quel que soit le niveau de résistance). Le TARV de troisième intention pour les adultes et les enfants est composé de darunavir boosté, de dolutégravir / raltégravir (le raltégravir a été utilisé entre 2015 et 2017 avant la disponibilité du dolutégravir) et parfois de deux N(t)RTI supplémentaires (sur la base des résultats du test de RMHIV). Nous avons choisis d'évaluer la

distribution des mutations contribuant à la résistance aux médicaments anti-VIH et leurs facteurs de risque chez les patients en échec de TARV de 2eme intention, et de déterminer les résultats de traitement chez les patients recevant un TARV de 3eme intention au Zimbabwe.

Les méthodes

Ce travail a été effectué en collaboration avec le ministère de la Santé du Zimbabwe dans le cadre du programme SIDA et tuberculose. Quatre centres nationaux de troisième intention ont été créés afin de fournir un traitement aux patients dont le traitement de deuxième intention échoue. Une procédure opératoire nationale et normalisée pour la prise en charge des patients en échec de TARV de deuxième intention a été élaborée. Dans l'un des centres de traitement, la Clinique de Newlands, les patients en échec de TARV de deuxième intention ont été inscrits dans un groupe de soutien à l'adhérence du traitement qui s'est réuni une fois par semaine (2,5 heures) pendant au moins six semaines. Le test de RMVIH a été effectué pour les patients suspectés en échec de traitement de deuxième intention, avec une charge virale confirmée> 1000 copies / ml, après 6 semaines de soutien amélioré à l'adhérence, et qui avaient une bonne adhérence du traitement, conformément aux directives nationales. Les séquences ont été analysées à l'aide du programme de la base de données des résistances aux médicaments anti-VIH de l'Université de Stanford, version 8.3. Les résultats du traitement de troisième intention ont été évalués à l'aide de mesures de charge virale, de décès et de pertes de suivi.

Résultats

Dans notre première publication: Sur un total de 186 participants qui ont reçu un soutien à l'adhérence thérapeutique suite à l'échec du traitement de deuxième intention, 61 ont atteint des charges virales de moins de 1000 copies / ml, 3 ont été perdus de vue, 1 a été transféré et 35 ne répondaient pas aux critères cliniques de génotypage en raison d'une mauvaise adhérence thérapeutique. Sur les 86 patients qui ont subi un test de RMVIH,

seulement 50 (58%) ont présenté des mutations majeures des inhibiteurs de protéase. Parmi les 36 patients qui ont reçu un TARV de troisième intention, les premiers résultats du traitement ont été excellents, 29 (81%) ayant atteint une charge virale <50 copies / ml à la semaine 24.

Dans notre deuxième publication: Sur un total de 209 patients qui avaient déjà reçu un TARV de troisième intention: 124 provenaient de la Clinique Newlands (CN) et les 85 restant des trois cliniques gouvernementales. Les résultats du génotype du VIH étaient disponibles pour 89 (72%) patients à la CN et 14 (16,5%) patients dans les cliniques gouvernementales. La durée médiane du TARV de troisième intention (en années) dans les cliniques gouvernementales était respectivement de 2,3 (IQR: 0,6-3,4), 1,3 (IQR: 0,7-1,7) et 1 (0,6-1,9). Sur les 67 patients qui ont reçu un TARV de troisième intention dans les cliniques gouvernementales pendant au moins six mois, 53 (79%) avaient la charge virale (CV) la plus récente <1000 copies / ml. Les données sur les autres résultats de traitement des cliniques gouvernementales étaient incomplètes. A la CN, 109 (88%) patients étaient toujours pris en charge, 13 (10,5%) étaient décédés et 2 (1,5%) avaient été transférés. La durée médiane du TARV de troisième intention était de 1,4 an (IQR: 0,6-2,8). Parmi les 111 patients CN qui avaient reçu un TARV de troisième intention pendant au moins 6 mois, 83 (75%) avaient une CV <50 copies / ml et 106 (95,5%) avaient une CV <1000 copies / ml.

Conclusion

Nos résultats démontrent qu'une mauvaise adhérence thérapeutique est la principale cause d'échec du traitement antirétroviral de deuxième intention. De plus, les patients dont le TARV de deuxième intention échoue peuvent atteindre des taux élevés de suppression virologique sous TARV de troisième intention. L'accès au génotypage du VIH au Zimbabwe est faible et peut constituer un obstacle à un diagnostic efficace de l'échec du TARV de deuxième intention et à des passages inappropriés au TARV de troisième intention.

Introduction

HIV Global Epidemic

The first case of Human Immunodeficiency Virus (HIV) infection was reported in San Francisco of the United States of America in 1981 [1] and the causative agent was discovered and named in 1983 [2]. The syndrome of HIV infection was named Acquired Immunodeficiency Syndrome (AIDS). Since its discovery, HIV/AIDS has devasted communities world-wide and the center of the global epidemic has been sub-Saharan Africa. The epidemic in Africa disproportionally affects women more than men and the virus is mainly transmitted during heterosexual intercourse. In other parts of the world outside Africa, the vast majority of epidemics are among predominantly male key populations (such as people who inject drugs and gay men and other men who have sex with men). The epidemic has reversed life expectancy in many countries, overburdened health delivery systems and severely hampered productivity and economic development. Although the rate of new infections is decreasing, the total number of people living with HIV in the world continues to increase due to the success of treatment programs. In 2017, the total number of people living with HIV reached 36.9 million (31.1 million – 43.9 million) and the majority of these i.e. 19.6 million (17.5 million-22 million) are living in eastern and southern Africa [3]. The number of AIDS related deaths is at its lowest ever, with less than 1 million people dying every year from AIDS-related conditions. This has been due to the increased access and effectiveness of antiretroviral therapy (ART) [3]. In 2014, the Joint United Nations Programme on HIV/AIDS (UNAIDS) and partners launched the very ambitious 90–90–90 targets; the aims of these targets were to diagnose 90% of all HIV-positive persons, initiate ART for 90% of those diagnosed, and achieve HIV viral suppression for 90% of those on treatment by 2020 [4]. Achieving 90% sustained viral suppression among people receiving ART is key to maximize individual health and survival and to reduce the incidence of HIV in the population.

HIV is an enveloped retrovirus belonging to the genus of lentiviridae, encasing in its core single-stranded ribonucleic acid (RNA), structural proteins and three replicative enzymes (reverse transcriptase, integrase and protease). The virus is subdivided into two types: HIV-1 which is the most common globally, and HIV-2, a less pathogenic variant found mostly in West Africa. HIV-1 has been subdivided into four separate genetic groups: M,N,O and P [5]. Of these groups, group M (major) is responsible for over 90% of infections globally. Group M has been further sub-classified into nine subtypes (A-D, F-H,J and K) and many circulating recombinant forms [6]. Subtype B is most prevalent in Europe, North America and Australia. Subtype C is responsible for 56% of infections in sub-Saharan Africa [7].

HIV Epidemic in Zimbabwe

Zimbabwe has one of the highest adult HIV prevalence in sub-Saharan Africa at 13.3 % with an estimated 1.3 million people living with HIV [3]. HIV epidemic is geographically diverse, ranging from a prevalence of 23.0% in Bulilima district in Matebeleland South region to approximately 9.3% in Gokwe North in Midlands region. According to 2017 HIV estimates, the HIV incidence in Zimbabwe was 0.541.The incidence varies by province with Matabeleland South having the highest incidence of 0.9 while Manicaland has the lowest incidence of 0.29 *[Zimbabwe Global AIDS 2018 Report]* The Zimbabwe national HIV epidemic is generalized and is mainly driven by unprotected heterosexual intercourse. Women are disproportionately affected, particularly adolescent girls and young women. However, there are growing epidemics among key populations and vulnerable groups such as sex workers, long distance truck drivers, prisoners and men who have sex with men who are at higher risk of HIV infection. National data on these populations are sparse as only a minimal amount of data are collected and reported in national documents. In 2017, new infections dropped to 41,000 from 79,000 in 2010, with behaviour change communication, high ART coverage and prevention of mother-to-child transmission

(PMTCT) services thought to be responsible for this decline. Deaths from AIDS-related illnesses continue to fall – from 61,000 in 2013 to 22,000 in 2017 *[Zimbabwe Global AIDS 2018 Report]*. According to UNAIDS 2018 data, 85% of Zimbabweans living with HIV knew their status and 95% of these were on ART [3]. The proportion of patients with suppressed viral load could not be established due to poor access to viral load testing. In 2016, Zimbabwe adopted a 'treat all' policy towards ART, meaning all people should be started on treatment immediately, regardless of their CD4 count as advised by WHO [8].

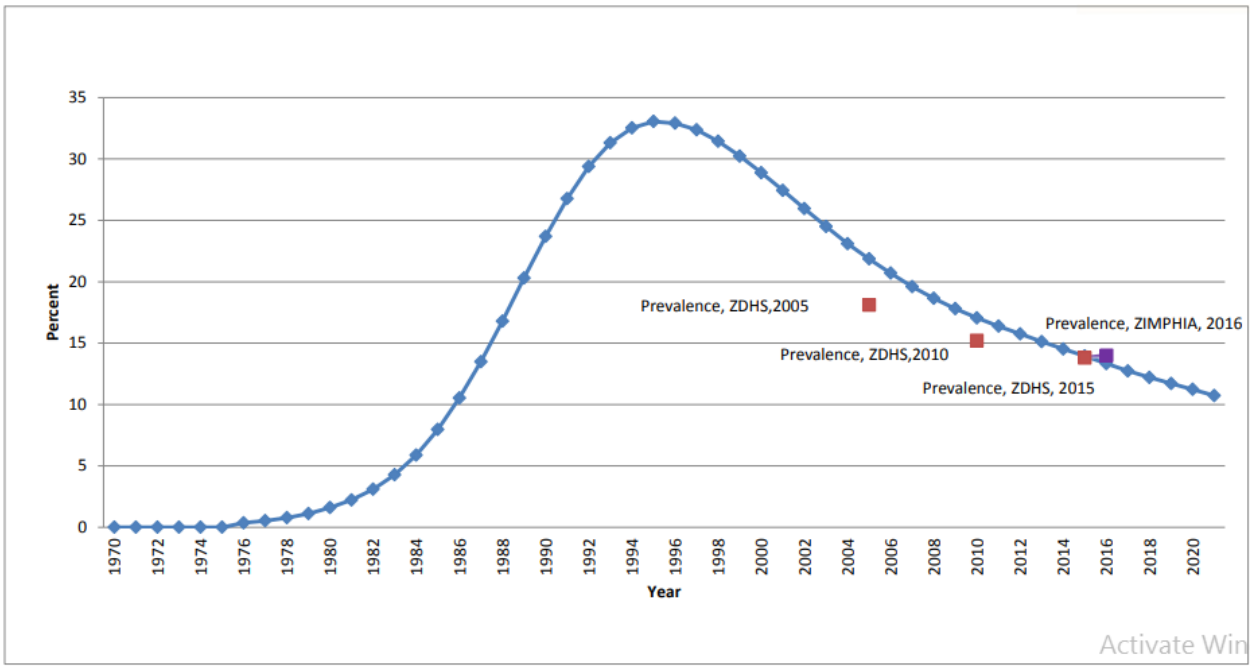
Table 1: Trends in estimated Number of People Living with HIV & AIDS in Zimbabwe

Population	**2014**	**2015**	**2016**	**2017**
Adults and Children	1,285,205	1,302,105	1,315,883	1,325,823
Adults (> 15 years of age)	1,194,760	1,216,615	1,234,982	1,249,172
Adolescents (10-19 years)	85,099	81,552	77,943	74,460
Adults (15-49 years)	1,005,850	1,014,440	1,016,238	1,014,395
Females (> 15 years)	703,467	717,172	728,927	738,399
Pregnant women (15-24 years)	16,160	15,800	15,320	14,816
Children (0-14 years)	90,445	85,489	80,902	76,650

Source: Zimbabwe-HIV-Estimates report 2018

Zimbabwe HIV Epidemic Curve

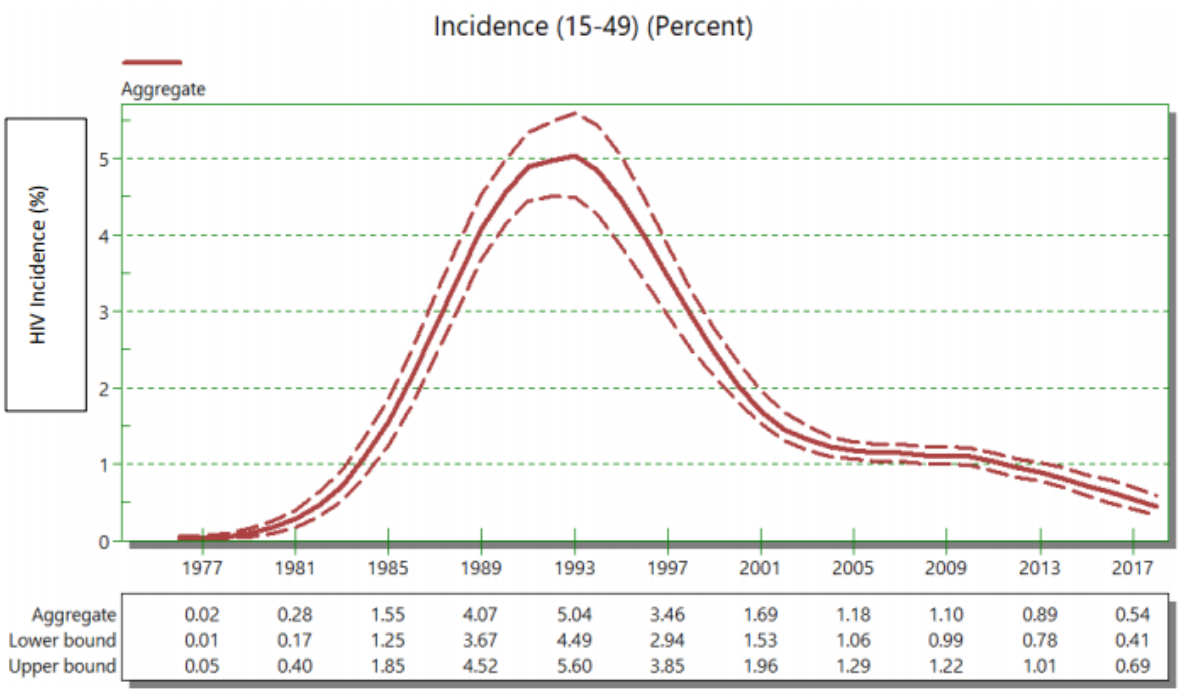
Figure 1: Trends in Adults (15-49 years) HIV Prevalence with ZDHS data points*



*****Zimbabwe Demographic Health Survey: ZIMPHIA= Zimbabwe Population-based HIV Impact Assessment

Source (Zimbabwe national and sub-national HIV estimates report 2017)

Figure 2: *Trends in Adults (15-49 years) HIV Incidence*



Source (Zimbabwe national and sub-national HIV estimates report 2017)

Antiretroviral Treatment

Since its introduction in 1996, treatment with ART has increased the life-expectancy of people living with HIV. ART reduces HIV-related morbidity and mortality by lowering the viral load to minimum levels, thereby allowing the immune system to improve and prevent the occurrence of opportunistic infections [9]. There currently exists no cure for HIV. The classification of ART medication is based on where in the viral replication cycle the medicines work. African countries began the roll out of ART in public institutions in 2002 and a large scale-up of access to ART across the continent has been achieved because of high levels of political commitment among governments and substantial international funding. In 2017, 21.7 million [19.1 million–22.6 million] people living with HIV were accessing antiretroviral therapy, up from 8 million [7.1 million–8.3 million] in 2010 [3].

To support the scale-up of ART programs in resource limited settings, a public health approach was developed by the World Health Organization (WHO). This approach uses simplified treatment protocols which include standard first line and second line ART regiments to facilitate effective care delivery in settings with limited clinical expertise and poor access to ART and laboratory monitoring [10]. Furthermore, the approach allows for limited laboratory monitoring and decentralized service delivery. This is in contrast to the individualized, specialist care in the resource rich settings of developed countries, that includes routine plasma viral load monitoring, drug resistance testing and a wide choice ART medicines. Prior to 2019, standard first line regimens consisted of a nucleoside reverse transcriptase inhibitor (NNRTI) and dual nucleoside / nucleotide reverse transcriptase inhibitors ($N_{(t)}$RTI) backbone available in most countries as fixed dose combinations. Standard second line regimens consisted of a protease inhibitor (PI) and a dual $N_{(t)}$RTI backbone [8]. In 2019, most countries have begun the transition to dolutegravir based first line ART following guidance from WHO. In the absence of routine virological monitoring, the diagnosis of treatment failure is based on clinical status and / or CD4 cell count. Most countries in sub-Saharan Africa have introduced routine virological monitoring, however, access to viral load testing remains low [11]. As part of strategy to eliminate transmission of HIV from mothers to children, the WHO recommends the administration of ART to HIV infected women and a short course of ART prophylaxis to their infants [8]

HIV Drug Resistance

HIV has mechanisms that ensure rapid viral evolution and hence characterized by high genetic diversity of the virus. The reverse transcriptase of HIV lacks proofreading activity and is therefore error-prone, introducing an average of one mutation for each genome transcribed [12]. Furthermore, HIV is characterized by high viral turnover with a production of an estimated 10 billion virions per day [13]. As a result, there is a large pool of genetic virus variants, called

quasispecies which are distinct but genetically related within an infected individual. In the absence of ART, the many genetic HIV variants in an infected individual will exist as minor populations only. Under selective pressure of ART medicines, certain variants may have an evolutionary advantage hence overgrowing the wild-type virus. Quasispecies may have mutations that either reduce their fitness or mutations that provide a fitness advantage in the presence of antiretroviral medicines. Mutations can cause drug resistance by structural alterations of the target molecule that will prevent or reduce inhibitor (ART medicine) binding, or by directly affecting the mechanism of action of the reverse transcriptase enzyme. In patients receiving antiretroviral therapy, the emergence of HIV drug resistance is possible only if the virus continues to replicate in the presence of sub-optimal drug levels that are insufficient to completely block viral replication but enough to exert a selective pressure on variants with decreased drug susceptibility. Many factors such as poor adherence, drug-drug interactions and medicine stock outs are drivers of HIV drug resistance. Drugs requiring accumulation of multiple mutations to overcome antiviral activity like zidovudine, dolutegravir and the protease inhibitors have a high genetic barrier to resistance. On the other hand, drugs requiring a single point mutation to confer high- level resistance like nevirapine and efavirenz have a low genetic barrier to resistance.

The term acquired or secondary HIV drug resistance is used to describe resistance that occurs in persons who are receiving ART. When individuals are newly infected by a drug resistant virus, the term transmitted, or primary HIV drug resistance is used. The term pretreatment drug resistance defines HIV drug resistance detected in people with HIV before they start ART, resulting from either previous exposure to antiretroviral drugs or transmission of a drug-resistant strain [14]. The rise in HIV resistance to NNRTIs in many low- and medium-income countries poses a growing threat of undoing the major gains of national ART programs [15].

There are several factors contributing to the emergence of HIV drug resistance in Africa, these can be broadly categorized into four groups: regimen- and drug specific, virus-related, patient specific and program related factors. Prevention, monitoring and response to HIV drug resistance is key to building and sustaining gains in HIV treatment scale-up, and achieving the global 90-90-90 targets for treatment [14]. The WHO has responded to the increasing threat of HIVDR by launching the Global Action Plan (GAP) on HIV drug resistance in 2017 [16]. The GAP outlines a framework for action to minimize the emergence and transmission of HIVDR so as to ensure effective treatment of HIV infected people. GAP has five strategic objectives which are as follows:

1. *Prevention and response*: Implement interventions to prevent and respond to HIVDR
2. *Monitoring and Surveillance:* Standardized surveys to assess pretreatment and acquired resistance in adults and children with the aim of obtaining nationally representative estimates.
3. *Research and innovation:* Encourage relevant research and innovation that will have impact on minimizing HIVDR
4. *Laboratory Capacity:* Increase capacity to perform viral load monitoring and ability to do HIVDR testing
5. *Governance and enabling mechanisms:* Ensure that governance and enabling mechanisms are in place to support action on HIVDR

Zimbabwe has put in place a robust HIV drug resistance surveillance programme. An HIV DR technical working group (TWG) is in place to support the Ministry of Health and Child Care (MOHCC) in monitoring the prevention of the development of HIV drug resistance. The MOHCC has been able to conduct Early Warning Indicator surveys annually. These surveys are a key component of WHO public health strategy to minimize and assess HIVDR in countries scaling up antiretroviral therapy [17]. In addition, pre-treatment drug resistance (PDR) and Acquired drug

resistance (ADR) surveys were conducted as per WHO guidance. The results of the PDR indicate an increasing proportion of ART naïve patients already have HIVDR, and this is above the WHO recommended threshold of 10% [18–20].

HIV drug resistance testing is currently not available in the public sector in Zimbabwe. Private laboratories send specimens for HIVDR testing to South Africa at a prohibitive cost of approximately six hundred Unites States dollars per test. The poor access to HIVDR testing in Zimbabwe is a significant barrier to the optimum care of patients failing second-line ART. Furthermore, there is scarcity of data in the country regarding trends of acquired HIV drug resistance. The emergence and transmission of HIV drug resistance in Zimbabwe and the region must be prevented, monitored and reversed. Every individual living with HIV must have access to effective lifelong ART.

Protease inhibitor (PI)-based ART

The number of patients receiving PI based second-line regimens in low- and medium-income countries (LMIC) has remained low (< 5% of all people on ART) despite the substantial rates of first-line failure [21]. Inadequate switching is due to multiple factors such as clinicians' inexperience, lack of viral load testing and unavailability of second-line drugs [22]. As access to viral load monitoring improves in sub-Saharan Africa, the number of people on protease inhibitor based second-line ART is expected to increase to 4-6 million people by 2030 [23].

Mutations that confer resistance to protease inhibitors are in the gene encoding the viral protease. Acquired resistance associated with protease inhibitors is infrequent among majority of patients who are viremic while receiving PI based second-line ART [24]. The rarity of PI associated mutations is mostly due to complete non-adherence (no mutational selective pressure) or mediation of PI resistance by mutations outside the protease gene, i.e. in the gag and env genes [25]. Clinical management of second-line failures is sub-optimal in many settings due to the

absence of resistance tests to identify people who harbor clinically relevant resistance mutations and need an optimized third-line regimen.

Zimbabwe National Antiretroviral Therapy Program

Zimbabwe is providing antiretroviral treatment to over a million people living with HIV. The success of the ART program has been made possible through strong commitment from the national government and the generous support received from development partners. There has been rapid expansion and decentralization of ART services. Over a million PLHIV are currently on ART, and over 1 500 ART Heath facilities are offering ART services. The country adopted "Treat All" strategy in 2016 and the strategy was rolled out nationwide in 2017.

A total of 1 150 079 patients were receiving ART in Zimbabwe as of 31 December 2018. The majority of these patients (78.5%) were enrolled at a primary care level facility: 4% of the patients were receiving second-line ART and only 202 patients were on third-line treatment as highlighted in the table 2 below.

Table 2: Distribution of patients receiving antiretroviral therapy in Zimbabwe in 2018

Output	Primary Care Level	District/Mission Hospital	Provincial Hospitals	Central Hospitals	Total
Patients in care	926 711	206 381	26 018	22 022	1 181 132
On ART	902 347	200 955	25 334	21 443	1 150 079
Patients on:					
First line	873 334	190 169	23 655	19 005	1 106 163
Second line	29 011	10 739	1 678	2 286	43 714
Third line	0	111	0	91	202

Source: Zimbabwe HIV & AIDS Estimates 2018 (National AIDS Council Report)

Following the WHO recommendations, Zimbabwe adopted a public health approach to its HIV program using standardized treatment regimens. Since the inception of the HIV treatment program in 2004, there have been several guideline changes in when to start patients on ART and what regimens to use. Adult first line regimens include Dolutegravir or NNRTI (Nevirapine or Efavirenz) plus two nucleoside / nucleotide reverse transcriptase inhibitors (N(t)RTIs). Second line regimens include a ritonavir boosted PI (Lopinavir or Atazanavir) and two N(t)RTIs. All patients receive triple therapy for the management of HIV infection except earlier in the program (2004-2015) when single dose nevirapine or nevirapine plus zidovudine dual combination was used for prevention of mother to child transmission (PMTCT) [26].

Third-line ART was introduced in Zimbabwe in 2015 through four designated national third line treatment centers. All the four centers are designated specialist HIV treatment clinics with access to laboratory and radiology support. Patients failing second line treatment should receive

at least three months of enhanced adherence support before a genotypic antiretroviral resistance test (GART) is done according to national guidelines. The Stanford HIV drug resistance database is used to assess resistance to ART. Second line treatment failure is confirmed by the presence of any level of resistance to atazanavir and lopinavir. Third-line ART is composed of boosted darunavir, dolutegravir and sometimes two additional N(t)RTIs (based on the GART results).

Monitoring for ART treatment success was predominantly done using clinical events and routine CD4 measurements. HIV RNA (viral load) measurements became available in the public sector in 2015 but to date limited number of patients have access to routine annual viral load (VL) measurements. First- and second-line regimen failure is diagnosed in a patient who has received the regimen for at least six months and has at least two VL results greater than 1000 copies /ml three months apart with good adherence to treatment.

Aim of Thesis

Zimbabwe has recently introduced third-line ART through the national program. To date no review has been done to document successes, challenges and outcomes of the provision of third line ART in the country. The aim of this thesis was to assess the extend and pattern of HIV drug resistance among patients failing second line ART. Furthermore, the thesis documents outcomes of third line ART in Zimbabwe.

The thesis highlights the challenges Zimbabwe is facing in managing HIV drug resistance and offers practical recommendations for the care of patients failing second-line ART. The thesis also raises awareness on the need to invest in strategies to care for patients failing third-line ART.

Methods

The work shared in this manuscript was done in collaboration with Zimbabwe Ministry of Health and Child Care as a response to the growing need for third-line ART in the country. The PHD student played a leading role in program design, training of health workers, data collection and analysis, manuscript writing and dissemination of results. The following were the major roles of the PHD student:

1. Engaging the national AIDS and Tuberculosis program on the need for provision of third-line ART in the public sector
2. Contributed to the drafting of the first national third-line ART protocol in the country
3. Developed the Newlands Clinic standard operating procedure for enhanced counselling among patients who failed second-line ART (See Appendix)
4. Initiated and participated in Newlands Clinic multidisciplinary team meetings were patients failing second-line ART were discussed to assess for eligibility for HIV genotyping and third-line ART

Article 1:

1. Developed the study protocol and obtained relevant ethical approvals
2. Through the assistance of the Institute of Social and Preventive Medicine (ISPM) of the University of Bern, developed a redcap database which was used to capture and analyze data for the first manuscript
3. Entered all patient level data into redcap
4. Interpretation of HIV drug resistance test results and the management of patients receiving third-line ART
5. Did the data analyses

6. Wrote the first draft of manuscript and with input from Supervisor and co-authors, revised the manuscript until publication
7. Presented results to local key stakeholders

Article 2

1. Engaged the Ministry of Health on the findings of the state of the third-line program in the country and obtained permission to evaluate the national program
2. Developed the protocol and obtained relevant ethical approvals
3. Developed data collection tool and collected data from all four third-line treatment centers
4. Did the data analysis
5. Wrote the first draft of manuscript and with input from Supervisor and co-authors, revised the manuscript until publication

Post-publication

I am now leading a taskforce to help the Ministry of Health and Child Care decentralize the provision of third-line ART across the country through training of healthcare workers in the different provinces

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MAJOR ARTICLE

HIV-1 Drug Resistance and Third-Line Therapy Outcomes in Patients Failing Second-Line Therapy in Zimbabwe

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Objectives. To analyze the patterns and risk factors of HIV drug resistance mutations among patients failing second-line treatment and to describe early treatment responses to recommended third-line antiretroviral therapy (ART) in a national referral HIV clinic in Zimbabwe.

Methods. Patients on boosted protease inhibitor (PI) regimens for more than 6 months with treatment failure confirmed by 2 viral load (VL) tests >1000 copies/mL were genotyped, and susceptibility to available antiretroviral drugs was estimated by the Stanford HIVdb program. Risk factors for major PI resistance were assessed by logistic regression. Third-line treatment was provided as Darunavir/r, Raltegravir, or Dolutegravir and Zidovudine, Abacavir Lamivudine, or Tenofovir.

Results. Genotypes were performed on 86 patients who had good adherence to treatment. The median duration of first- and second-line ART was 3.8 years (interquartile range [IQR], 2.3–5.1) and 2.6 years (IQR, 1.6–4.9), respectively. The median HIV viral load and CD4 cell count were 65 210 copies/mL (IQR, 8728–208 920 copies/mL) and 201 cells/mm^3 (IQR, 49–333 cells/mm^3). Major PI resistance-associated mutations (RAMs) were demonstrated in 44 (51%) non-nucleoside reverse transcriptase inhibitor RAMs in 72 patients (83%) and nucleoside reverse transcriptase inhibitors RAMs in 62 patients (72%). PI resistance was associated with age >24 years ($P = .003$) and CD4 cell count <200 cells/mm^3 ($P = .007$). In multivariable analysis, only age >24 years was significantly associated (adjusted odds ratio, 4.75; 95% confidence interval, 1.69–13.38; $P = .003$) with major PI mutations. Third-line DRV/r- and InSTI-based therapy achieved virologic suppression in 29/36 patients (81%) after 6 months.

Conclusions. The prevelance of PI mutations was high. Adolescents and young adults had a lower risk of acquiring major PI resistance mutations, possibly due to poor adherence to ART. Third-line treatment with a regimen of Darunavir/r, Raltegravir/Dolutegravir, and optimized nucleoside reverse transcriptase inhibitors was effective.

Keywords. HIV-1 drug resistance; second-line therapy; third-line ART outcomes; Zimbabwe.

The many benefits of combination antiretroviral therapy (ART) may be compromised by virologic failure and drug resistance [1]. ART programs in countries hard hit by the HIV pandemic in Sub-Saharan Africa are facing increasing virologic failure of firstline ART and high levels of drug resistance to non-nucleoside reverse transcriptase inhibitors (NNRTIs) [2]. The emergence of resistance to ART is a consequence of expanded access to treatment and longer duration of ART exposure. To maintain the benefits of ART, international guidelines recommend switching to second-line, boosted protease inhibitor (PI)–based ART to maintain virologic suppression [3]. Routine HIV viral load monitoring is essential for the early diagnosis of ART treatment failure [4]. In contrast to patients failing firstline NNRTI- and nucleoside reverse transcriptase inhibitor (NRTI)–based ART, the majority of patients failing with a PI-based second-line ART regimen do not acquire major PI resistance-associated mutations [5, 6].

As more people with suboptimal adherence are on ART, the number of patients failing first- and second-line ART regimens is increasing, and an increase in multiclass drug resistance is expected [7]. Ongoing success of ART programs will require an understanding of the emergence and patterns of HIV drug resistance among individuals in whom treatment has failed. Virologic failure occurs for multiple reasons, including suboptimal adherence and drug intolerance/toxicity leading to drug resistance. After second-line failure, evidence of multiclass resistance following exposure to boosted PI regimens requires treatment with at least 2 fully active antiretroviral drugs to suppress viremia, reduce the transmission of resistant virus, and optimize the effectiveness of third-line ART. Several factors, such as the duration of PI use and viral load, have been identified as risk factors for developing PI resistance mutations [8]. Modeling provides evidence that genotyping to optimize third-line ART is more cost-effective than switching patients failing

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second-line to third-line based only on virologic failure [9]. In the Zimbabwe National ART program, boosted darunavir, recycling available nucleoside/nucleotide reverse transcriptase inhibitors, and an integrase strand transfer inhibitor (InSTI) are provided, as recommended by the World Health Organization (WHO) [3]. However, identification of multiclass drug resistance and eligibility for third-line therapy requires genotypic resistance testing (GRT). For patients who have developed multiclass resistance to NNRTI, NRTIs, and boosted PIs, InSTIs have been approved as a new class of ART with a high barrier to resistance and an exceptional safety profile [10]. While recently approved in Botswana for firstline treatment, InSTIs are currently reserved for patients with evidence of multiclass resistance in most resource-limited countries, including Zimbabwe. Darunavir, a second-generation protease inhibitor, has been shown to be effective against HIV resistant to Atazanavir and Lopinavir, and hence is a useful third-line option [11, 12]. Third-line treatment should include new drugs with a minimum risk of cross-resistance to previously used regimens that are available in resource-limited settings [13].

In this study, we analyzed the patterns of HIV drug resistance mutations among patients failing second-line treatment and the risk factors for acquiring major PI resistance. We further describe early treatment responses to recommended third-line ART in an HIV clinic in Zimbabwe. Our broad aim was to inform planning for third-line ART programs in sub-Saharan Africa.

MATERIALS AND METHODS

Study Setting and ART Treatment Guidelines

Newlands Clinic is an HIV treatment center in Harare, Zimbabwe, that is a national referral site for patients who are supposed to start third-line ART treatment after second-line virologic failure. Firstline regimens comprise 2 nucleoside/NRTIs—among them tenofovir (TDF), zidovudine (ZDV), abacavir (ABC), and lamivudine (3TC), and an NNRTI, either efavirenz (EFV) or nevirapine (NVP). Until 2013, stavudine (D4T) was part of the national firstline ART regimen. Second-line regimens include 2 NRTIs and a ritonavir-boosted PI, either atazanavir or lopinavir. The NRTIs used in second-line are 3TC and AZT or TDF or ABC, depending on what the patient received for firstline treatment. Protease inhibitor monotherapy is not part of national guidelines, and none of the patients studied received it. National guidelines recommend a change to a third-line regimen if virologic failure (2 consecutive HIV RNA tests >1000 copies/mL) occurs after at least 6 months of therapy and adherence is estimated to be greater than 95% by pill counts and/or pharmacy refill records [14]. National guidelines recommend that patients on second-line ART have at least 1 viral load test done per year. Patients with an elevated VL (>1000 copies/mL) must have a repeat test done 3 months after adherence support. At Newlands Clinic only, patients who are deemed adherent (assessed by pill counts) after an intensive adherence support program and who have acquired major PI resistance mutations are commenced on third-line ART. Third-line regimens consist of Darunavir/ritonavir, Raltegravir or Dolutegravir, and an (optimized) NRTI based on GRT.

Enhanced Adherence Support Program

All patients suspected of second-line ART failure, that is, patients who had a VL >1000 copies/mL, were enrolled in a 6-week enhanced adherence support program before GRT between August 1, 2013, and July 31, 2016. Patients who had elevated viral loads met in support groups of 8–10 participants once weekly for approximately 2.5 hours. Group cognitive behavioral counseling was aimed at discussion of HIV and ART, the identification of barriers and challenges to adherence, and the strengthening of medication adherence. These meetings were facilitated by trained counselors. There were separate groups for participants age 24 years and younger and for those older than age 24 years. All patients had the VL test repeated after the adherence support program. HIV-1 viral load was measured by the Roche COBAS Ampliprep/COBAS Taqman HIV-1 Test, version 2.0.

ART Resistance Testing

GRT was done for patients suspected of second-line failure with a confirmed viral load >1000 copies/mL after 6 weeks of enhanced adherence support, and had good adherence, as per national guidelines. GRT was not done in patients who still had confirmed poor adherence after adherence support. Patients with poor adherence continued to receive adherence support, and repeated viral load tests were done every 3 months. Plasma viral RNA was extracted, reverse transcribed, and 1.3 kb of the HIV-1 protease and reverse transcriptase genes was amplified as described by Manasa [15]. Amplicons were sequenced at MCLab Molecular Cloning Laboratories (http://www.mclab.com), San Francisco, California. The chromatograms were assembled using Geneious software, version 8 (http://www.geneious.com) [16], and consensus sequences were analyzed using the Stanford University HIV Drug Resistance Database's HIVdb program, version 8.3 (https://hivdb.stanford.edu/hivdb/by-sequences) [17]. The estimated level of resistance to ART was determined by the genotypic susceptibility scores (GSS) associated with each of the drug resistance mutations. The estimated level of resistance was calculated as follows: susceptible (total score 0–9), potential low-level resistance (total score 10–14), low-level resistance (total score 15–29), intermediate resistance (total score 30–59), and high-level resistance (total score of 60 and above).

Data Analysis

We analyzed the clinical data that were routinely collected for each patient. We used univariable and multivariable logistic regression to study the association between explanatory variables with the development of at least 1 major PI resistance-associated mutation (RAM). We included the following explanatory variables: age (≤24 and >24 years according to the WHO, which

defines adolescents and young adults as people aged ≤24 years [18]), HIV RNA (≤100 000 and >100 000 copies/mL), sex, CD4 cell count (≤200 and >200 cells/mm^3), and duration of second-line therapy (≤2 and >2 years). All variables were retained in multivariable logistic regression regardless of association in univariable analysis. Statistical tests were 2-sided, with a significance level of .05. There were no missing values. All statistical analyses were performed in Stata, version 13.0 (StataCorp, College Station, TX).

Ethics

The study was approved by the Medical Research Council of Zimbabwe (approval No. MRCZ/A/1336). Patients provided written informed consent before being enrolled into the enhanced adherence support program.

RESULTS

A total of 186 participants received adherence support for second-line failure, 61 achieved postadherence support viral loads of less than 1000 copies/mL, 3 were lost to follow-up, 1 was transferred out, and 35 did not meet clinical criteria for genotyping due to confirmed poor adherence.

Of the 86 who were genotyped, 41 (48%) were female. Thirty-six (42%) had initiated firstline ART at Newlands Clinic and had been switched to a second-line regimen after failing first-line ART, and 50 patients (58%) were referred to Newlands Clinic, receiving second-line ART. The median age at genotyping was 27.7 years (IQR, 19.7–42.3 years). The median HIV viral load and CD4 cell count at the time of genotyping were 65 210 copies/mL (IQR, 8728–208 920 copies/mL) and 201 cells/mm^3 (IQR, 49–333 cells/mm^3), respectively. Participants had received firstline ART for a median of 3.8 years (IQR, 2.3–5.1 years) and second-line ART for a median of 2.6 years (IQR, 1.6–4.9 years). Participants had received a median of 6 (IQR, 6–7) antiretroviral medicines for first- and second-line ART. Table 1 summarizes participant demographic and clinical characteristics at the time of GRT. There were differences in education level (P = .006), CD4 cell counts (P = .032), HIV viral load (P = .039), and marital status (P = .001) between patients who had PI RAMs and those without. Only 2 participants received ART for the prevention of mother-to-child transmission; both had received single-dose nevirapine.

Drug Resistance–Associated Mutations

Sanger sequencing was successful for all 86 patients. All patients had subtype C virus. Wild-type virus was found in 12 (14%) participants, and 74 (86%) had mutant virus. Most (n = 72, 83%) had at least 1 NNRTI RAM, as summarized in Figure 1. The most common NNRTI mutation was K103N (n = 30, 35%), followed by Y181C (n = 26, 32%) and G190 (n = 24, 28%).

Sixty-two participants (72%) had at least 1 NRTI RAM. The distribution of major NRTI mutations is summarized in Figures 1 and 2. Two-thirds had the NRTI mutation M184V (n = 58, 67%), followed by the thymidine analogue mutations T215Y (n = 31, 36%) and D67N (n = 31, 36%). Overall, 13 (15%) patients had the K65R mutation, which confers high-level resistance to Tenofovir. All 13 patients with the K65R mutation had been exposed to TDF for either first- or second-line ART.

Table 1. Sociodemographic, Clinical, and Biological Characteristics of Study Population With HIV-1 Sequences (n = 86) Comparing Those With and Without PI Mutations

Parameter	All Patients (n = 86)	No PI Mutation (n = 42)	Any PI Mutation (n = 44)	*P* Value
Median age (IQR), y	27.7 (19.7–42.3)	21.2 (18.0–38.3)	37.4 (25.9–46.9)	.004
Gender, n (%)				
Female	41 (47.7)	22 (52.4)	19 (43.2)	.729
Marital status, n (%)				
Single	47 (54.7)	32 (76.2)	15 (34.1)	.001
Married	30 (34.9)	7 (11.7)	23 (52.3)	
Widowed	7 (8.1)	3 (7.1)	4 (9.1)	
Divorced	2 (2.3)	0 (0)	2 (4.6)	
Level of Education				
None	12 (14)	9 (21.4)	3 (6.8)	.006
Primary	17 (19.7)	12 (28.6)	5 (11.4)	
Secondary	43 (50)	18 (42.9)	25 (56.8)	
Tertiary	14 (16.3)	3 (7.1)	11 (25)	
Clinical				
CD4 count, median (IQR), cell/mm^3	201 (49–333)	243 (132–379)	97 (22–277)	.032
HIV RNA, median (IQR), copies/mL	65 210 (8728–208 920)	37 238 (4620–147 592)	79 362 (20 376–254 612)	.039
Duration of ART (IQR), y	7.7 (5.3–9.4)	7.3 (5.0–9.4)	7.9 (6.1–9.5)	.388
2nd-line ART duration (IQR), y	2.6 (1.6–4.9)	2.4 (1.6–4.7)	2.6 (1.7–5.2)	.318
No. of ART drugs received, median (IQR) at GRT	6 (6–7)	6.5 (6–8)	6 (5–7)	.081

Abbreviations: GRT, genotypic resistance testing; PI, protease inhibitor; RAM, resistance-associated mutation.

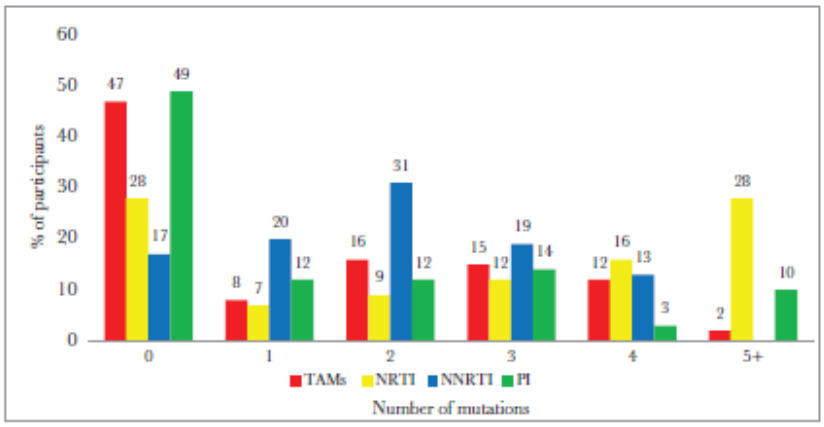

Figure 1. Distribution of HIV drug resistance mutations (n = 86). Abbreviations: NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; TAM, thymidine analogue mutation.

At the time of GRT, of the patients with the K65R mutation, 4 were receiving TDF/3TC, 2 AZT/3TC, and 7 ABC/3TC, as the NRTI backbone for second-line treatment. The multidrug NRTI drug resistance mutations T69Ins and Q151M were present in 7 (8%) and 4 (5%) participants, respectively. Overall, 46 (54%) had at least 1 thymidine analogue mutation (TAM), and 25 (30%) had 3 or more TAMs. Figure 1 highlights the observed frequency of TAMs among the participants; 66 (76.7%) participants had pathway 2 TAMs [19].

PI RAMs were present in 50 (58%) participants, and major PI RAMs were present in 44 (51%) participants. Figures 1 and 2 summarize the frequency of major PI mutations and the

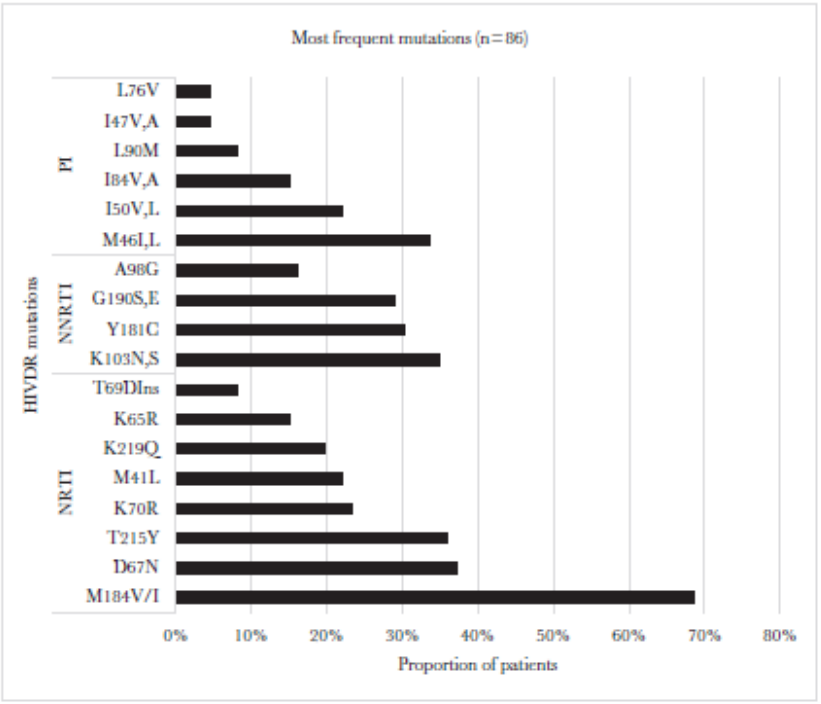

Figure 2. Distribution of specific HIV drug resistance mutations. Abbreviation: HIVDR, HIV drug resistance.

number of major PI mutations per patient. The most common PI mutation was M46I (n = 28, 33%), followed by I50V (n = 18, 21%) and V82A (n = 18, 21%). A total of 24 participants (28%) had at least 3 major PI RAMs.

Figure 3 highlights the results of estimated resistance for potential third-line ART drugs. The HIV drug resistance interpretation for PI showed that 40 (48%) participants had a virus susceptible to atazanavir, 62 (74%) were fully susceptible to darunavir, and 44 (52%) were fully susceptible to lopinavir. For NNRTI, full susceptibility was predicted for 18 (21%) to nevirapine, 19 (23%) to efavirenz, 21 (25%) to etravirine, and 19 (22%) to rilpivirine. For the NRTIs, full susceptibility to Lamivudine/Emtricitabine was noted in 26 (31%), to Abacavir or Didanosine was noted in 25 (30%), to Zidovudine was noted in 44 (51%), and to Tenofovir was noted in 35 (42%).

Risk Factors for PI Drug Resistance Mutations

Table 2 summarizes the risk factors for developing PI mutations. In univariable analysis, participants who had a CD4 cell count of <200 cells/mm^3 (odds ratio, 3.67 cells/mm^3; 95% confidence interval [CI], 1.43–9.43 cells/mm^3) were more likely to have major PI mutations. Age >24 years was independently associated with the risk of having major PI mutations in multivariable analysis (adjusted odds ratio, 4.75 years; 95% CI, 1.69–13.38 years). HIV viral load and CD4 cell count were not independently associated with the risk of having major PI mutations; neither was the duration of receiving PI-based second-line ART.

Early Third-Line Outcomes

The decision to switch to third-line ART was based on the presence of major PI RAMs conferring resistance to Atazanavir and Lopinavir. A total of 36 patients (19 females and 17 males) were commenced on a third-line ART regimen of darunavir, raltegravir, and optimized NRTI. Two patients with PI mutations continued on second-line (1 had very poor adherence due to psychiatric illness, and the other was receiving palliative care for disseminated cancer of the cervix), and 6 patients died before commencing third-line ART. Figure 4 highlights the outcomes of the patients who received third-line therapy. The median age of patients at commencement of third-line therapy was 41 years (IQR, 30–47.5 years). Patients were severely immunosuppressed with a median CD4 cell count of 147.5 cells/mm^3 (IQR, 28–252.5 cells/mm^3) and a median HIV viral load of 57 774 copies/mL (IQR, 18 809–215 624 copies/mL) at commencement of third-line therapy. At the time of analysis among participants commenced on third-line ART, none had been lost to follow-up and 2 had died, 1 due to chronic renal failure (diagnosed while the participant was on firstline therapy) and 1 due to acute alcohol-induced pancreatitis.

At week 24 on third-line therapy, the median CD4 cell count increased from 147.5 to 251.5 cells/mm^3 (IQR, 187.5–381 cells/mm^3). At week 24 on third-line therapy, 29/36 (81%) participants achieved viral suppression of <50 copies/mL, 5/36 (14%) patients had VL between 50 and 1000 copies/mL and 1/36 (3%) had died. One, a 17-year-old adolescent, had a week 24 VL of 2244 copies/mL and has been receiving adherence support, and to date he has not managed to achieve virological suppression. There were no reported discontinuations due to toxicity of any of the third-line medicines.

Among the 39 patients who had no PI mutations and continued on second-line ART with ongoing adherence support, only 8 achieved virological suppression 24 weeks after HIV drug resistance testing. Two participants were recommenced on firstline (because they had wild-type virus), and both achieved virological suppression after 24 weeks of firstline ART.

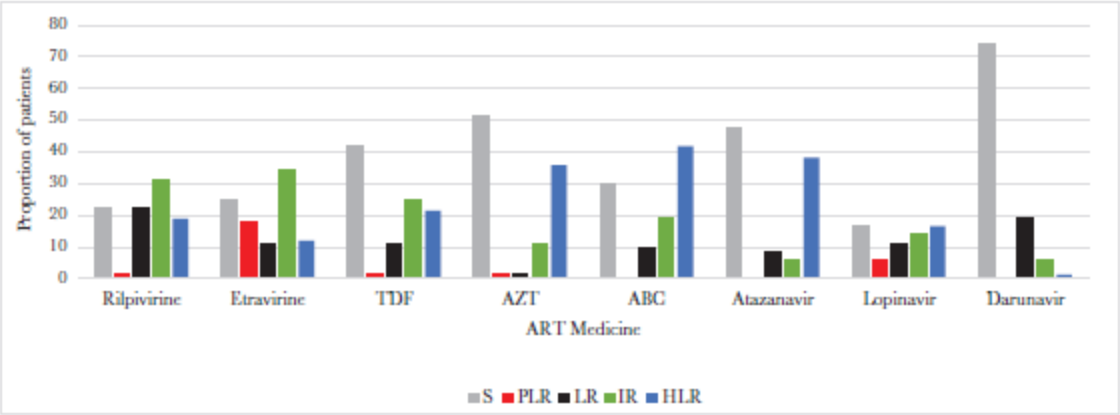

Figure 3. HIV drug resistance interpretation. Abbreviations: ABC, Abacavir; AZT, Zidovudine; HLR, high-level resistance; IR, intermediate resistance; LR, low-level resistance; PLR, potential low-level resistance; S, susceptible; TDF, Tenofovir.

Table 2. Risk Factors for Major Protease Inhibitor Resistance Mutations

	Univariable		Multivariable	
Risk Factor	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value
VL > 100 000 copies/mL	2.04 (0.84–4.92)	.114	2.14 (0.75–6.12)	.155
2nd-line duration > 2 y	1.31 (0.5–3.14)	.541	1.65 (0.61–4.50)	.327
Age > 24 y	4.11 (1.62–10.43)	.003	4.75 (1.69–13.34)	.003
CD4 < 200 cells/mm^3	3.67 (1.43–9.43)	.007	2.53 (0.90–7.15)	.079

Abbreviations: CI, confidence interval; OR, odds ratio; VL, viral load.

DISCUSSION

Among patients referred for second-line failure and genotyped after 6 weeks of aggressive adherence support, 14% had wild-type virus, suggesting very low adherence, and 86% had mutant virus. Among those with drug resistance mutations, all had 1 or more NNRTI and/or NRTI mutations and 44/86 (51%) had major PI resistance mutations. Younger patients (<24 years), were less likely to have acquired major PI drug resistance mutations upon failing PI-based second-line treatment. Viral load and immunological status at resistance testing were not independently associated with major PI RAMs. Early third-line treatment outcomes were excellent, with 30/36 patients achieving viral loads <50 copies/mL at 24 weeks.

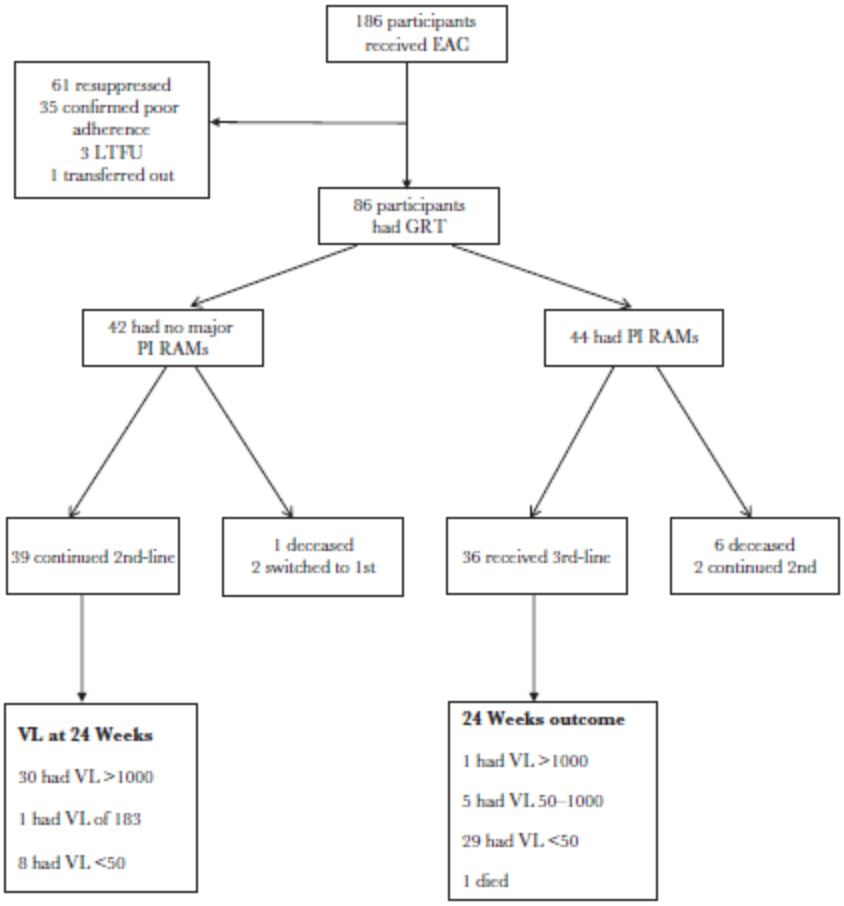

Figure 4. Outcomes of patients failing second-line antiretroviral therapy who received genotypic resistance testing. Abbreviations: EAC, enhanced adherence counseling; GRT, genotypic resistance testing; LTFU, loss to follow-up; PI, protease inhibitor; RAM, resistance-associated mutation; VL, viral load.

This study has some limitations. Our sample size was small. However, as resistance data after second-line failure are scarce, we believe that these results are important to clinicians looking after patients failing second-line ART. Population-based sequencing, used in this analysis, is not able to detect minority resistant viral strains, thus potentially underestimating resistance [20]. Moreover, the durability of suppression on third-line therapy was only established for 6 months, and longer follow-up is essential. Lack of resistance patterns after firstline failure was also a limitation.

Patterns of DRM demonstrate that despite interventions to improve adherence, almost half of the patients who had GRT done did not acquire major PI resistance-associated mutations. A total of 61 (33%) patients out of the original 186 resuppressed after adherence support, highlighting that they had a virus susceptible to second-line ART. This provides evidence that virologic failure is likely due to poor adherence, leading to reduced drug exposure. A number of studies from resource-limited settings have reported low rates of PI resistance after failure of second-line ART [5, 6, 21], often attributing this finding to poor medication adherence. The presence of major protease inhibitor mutations at the time of second-line failure ranges from 0% to 50% [22]. A recent national survey in Kenya reported a 25% prevalence of PI mutations among patients failing second-line ART [23]. The high prevalence of PI resistance in our cohort can be attributed to possible selection bias. Only patients with reported good adherence (after at least 6 weeks of enhanced adherence counseling) had a GRT done, that is, only 86 out of the original 186. The association of younger age and PI resistance is consistent with findings from similar studies in South Africa and the United Kingdom [5, 24, 25]. These studies show that second-line failure in young people is often due to poor adherence rather than development of PI RAMs. Age may provide an explanation for some of the patient-level, regimen-specific, and structural factors associated with the absence of PI mutations and reduced adherence to second-line ART [26–29]. Social and structural obstacles to adherence can include inaccessible clinic location or lack of access to transportation, work/child care responsibilities, and low health care provider to patient ratio as a consequence of the rapid growth in ART rollout programs [30, 31]. Optimizing treatment adherence and retention at all stages in the cascade of HIV care is critical to the prevention of resistance.

As expected in Africa, the predominant NRTI mutation observed in our cohort was MI84V, which confers high-level resistance to Lamivudine and Emtricitabine [32]. The observed prevalence of TAMs was high, the commonest being T215Y and D67N. TAMs are known to accumulate in patients who remain on a failing ART regimen due to delays in detecting treatment failure [33]. Patients may have been failing on second-line ART for a long time before enrolling in the adherence program, but either viral loads were not done prior to that time or the data were not provided. It is important for HIV treatment programs to offer routine viral load testing to enable early diagnosis of treatment failure and hence prevent accumulation of TAMs. TAMs have the potential to confer resistance to all drugs in the NRTI class.

Despite the absence of NNRTI exposure during second-line ART, the virus from almost 84% of patients had at least 1 major NNRTI resistance mutation, and the virus from 40% had a mutation at the K103N codon, strongly associated with resistance to nevirapine and efavirenz. The persistence of NNRTI resistance is consistent with genotypic analyses of 2 South African cohorts that failed PI-based ART [5, 25] and precludes recycling of firstline NNRTI drugs in third-line therapy. The high levels of NNRTI resistance mutations may indicate extensive drug resistance including NRTI mutations prior to the onset of second-line therapy.

The need for evidence regarding the implementation of third-line ART in resource-limited settings has been recognized by the WHO [3]. Our data demonstrate the effectiveness of third-line ART in a cohort of patients who are infected with HIV subtype C. The majority of patients achieved virologic suppression on regimens including darunavir/ritonavir, raltegravir, and NRTIs, suggesting that this can be used as a standardized third-line regimen in Zimbabwe. Data on the treatment outcomes of third-line ART are still very scarce in sub-Saharan Africa; however, 2 other reports have provided evidence of effectiveness [34, 35]. In a small Indian cohort, early treatment outcomes showed excellent effectiveness of third-line ART [36]. Although the small cohort size limits wider assumptions of efficacy, the preliminary outcomes suggest that third-line therapy can be effectively implemented in a resource-limited setting with excellent rates of virologic suppression. Furthermore, our results support the use of darunavir/ritonavir and an InSTI backbone for third-line ART, as recommended by the WHO [3].

CONCLUSIONS

Prevalence of RAMs was high among participants failing second-line ART. However, only half of these participants had major PI RAMs, which necessitate the switch to third-line treatment. The presence of major PI RAMs was significantly associated with an increase in age. Younger participants were more likely to fail second-line treatment due to poor adherence rather than development of PI resistance. GRT is essential to identify those with triple class resistance, and those who require third-line therapy to regain and sustain virologic suppression. A Darunavir/r, Integrase strand transfer inhibitor and optimized NRTI (based on GRT) regimen was effective in achieving virologic suppression in early follow-up. Our results show that third-line regimens for patients with multidrug-resistant HIV in Africa are likely to be effective.

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RESEARCH ARTICLE

Zimbabwe's national third-line antiretroviral therapy program: Cohort description and treatment outcomes

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Abstract

Background

In 2015, Zimbabwe introduced third-line antiretroviral therapy (ART) through four designated treatment centers; three government clinics in Harare and Bulawayo, and Newlands Clinic (NC), operated by a private voluntary organization in Harare. We describe characteristics of patients receiving third line ART and analyzed treatment outcomes in this national programme as of 31 December 2018.

Methods

We described the population using proportions for categorical variables, and medians and interquartile ranges for continuous variables. Patients from NC, where data were more complete, were followed from the date of starting third-line ART until death, transfer, loss to follow up or 31 December 2018.

Results

A total of 209 patients had ever received third-line ART: 124 at NC and 85 from the three government clinics. HIV genotype results were available for 89 (72%) patients at NC and fourteen (16.5%) patients in the government clinics. Median duration of third line ART (years) in the government clinics was 2.3 (IQR:0.6–3.4), 1.3 (IQR: 0.7–1.7) and 1 (0.6–1.9). Of the 67 patients who received third line ART in the government clinics for at least six months, 53 (79%) had most recent viral load (VL) < 1000 copies/ml. Data on other treatment outcomes from government clinics were incomplete.

From NC: a total of 109 (88%) patients were still in care, 13 (10.5%) had died and 2 (1.5%) were transferred. Median duration of third-line ART was 1.4 years (IQR: 0.6–2.8). Among the 111 NC patients who had received third-line ART for at least 6 months, 83 (75%) had a VL <50 copies/ml and 106 (95.5%) had a VL <1000 copies/ml.

Conclusion

Our findings demonstrate that, with comprehensive care, patients failing second-line ART can achieve high rates of virological suppression on third-line regimens. There is need to decentralize the provision of third-line ART in Zimbabwe. More needs to be done to improve completeness of data in the government clinics.

Background

Zimbabwe has one of the highest burdens of HIV infection in the world with an adult prevalence of 14.2% and approximately 1.4 million people living with the infection [2016 Zimbabwe HIV Estimates]. As the national programme continues to grow, it is expected that the numbers of patients failing current first-line non-nucleoside reverse transcriptase inhibitor (NNRTI)-based antiretroviral therapy (ART) and needing second-line protease (PI)-inhibitor-based ART will increase [1]. With more and more people receiving PI-based second-line ART, which is more difficult to adhere to due to the higher pill burden and more toxicity, we anticipate that the need for third-line ART will increase. While the availability of second-line ART is expanding in resource limited settings (RLS), only a few countries have widely available treatment options for patients who fail both NNRTI and PI-based combinations due to the high cost and complexity of implementation [2]. The World Health Organisation (WHO) has recommended that national programs must make third-line ART available [3]. Third-line ART regimens should include medicines such as etravirine (NNRTI), boosted darunavir (PI) and dolutegravir (Integrase inhibitor). Recommended approaches to third-line therapy require access to routine HIV viral load (VL) monitoring and genotypic resistance testing [3,4].

The provision of third-line ART in resource limited sub-Saharan Africa (SSA) has many challenges. Access to routine VL measurements among patients on ART is low and this leads to late diagnosis of ART treatment failure [5]. Previous studies have shown that the majority of patients failing second-line ART have not acquired protease inhibitor resistance mutations [6–8]. Poor adherence has been shown to be the major reason for second-line ART failure [9,10]. Unfortunately, access to HIV drug resistance (HIVDR) testing is poor and hence accurate diagnosis of second-line failure in SSA is difficult. Furthermore, before third-line commencement, patients should be adequately counselled on the need for optimum adherence and this requires substantial time which is not always available because of the high patient volumes and low numbers of healthcare workers.

Documentation of outcomes of third-line ART in RLS is scarce, yet very important to enable improvement of patient care. Due to the high costs of third-line regimens it is crucial to assess the effectiveness of these regimens to ensure optimal use of the limited resources. A few studies have reported encouraging early treatment outcomes for patients receiving third-line ART in RLS [8,11–13]. Results have shown that patients who switch to third-line ART have good early treatment outcomes and are able to suppress their VL despite the high level of ART resistance observed before third-line initiation [12]. Data on long term outcomes of over a year for patients receiving third-line ART are not yet available in sub-Saharan Africa.

We set out to describe the cohort of patients receiving third-line ART in Zimbabwe as well as assess treatment outcomes. We describe the following: demographic characteristics of patients, proportion of patients who were still in care, died and those lost to follow up. We further report on the virological outcomes among patients who had access to routine VL

monitoring at one of the main treatment centers. We have previously reported week 24 third-line treatment outcomes for patients receiving care at Newlands Clinic (NC) [8].

Methods

Study setting

The study was conducted at the four designated national third-line ART treatment centers. Three of the centers are government run opportunistic infections (OI) clinics and the fourth center, NC, is operated and funded by a private foundation. Three of these centers, (Newlands Clinic, Harare Central Hospital and Parirenyatwa Central Hospital) are in the capital city, Harare. The fourth center is in the second largest city, Bulawayo. All four clinics use standard national HIV treatment guidelines for the care of patients.

Unlike the other three centers, NC has had an effective electronic monitoring system from its inception. Furthermore, the clinic provides HIV genotyping, six monthly routine viral load monitoring, and other necessary laboratory and radiology services at no cost to the patient. Details of the model of care are published elsewhere [14].

Treatment regimens

Following the WHO recommendations, Zimbabwe adopted a public health approach to its HIV program using standardized treatment regimens. Since the inception of the HIV treatment program in 2004, there have been several guideline changes in when to start patients on ART and what regimens to use. Until beginning of April 2019, adult first line regimens included an NNRTI (Nevirapine or Efavirenz) plus two nucleoside / nucleotide reverse transcriptase inhibitors (N(t)RTIs). In April 2019, the preferred first-line regimen was changed to a dolutegravir-containing regimen as recommended by WHO. Second line regimens include a ritonavir boosted PI (Lopinavir or Atazanavir) and two N(t)RTIs. All patients received triple therapy for the management of HIV infection except earlier in the program (2004–2015) when single dose nevirapine or nevirapine plus zidovudine dual combination was used for prevention of mother to child transmission (PMTCT).

Third-line ART was introduced in Zimbabwe in 2015 through four designated national third-line treatment centers. All the four centers are designated specialist HIV treatment clinics with access to laboratory and radiology support. Patients failing second-line ART are transferred to the nearest third-line center by their treating physicians. These patients are supposed to receive at least three months of enhanced adherence support before being offered HIVDR testing. National standard operating procedures recommend that patients must have confirmed good adherence to second-line ART to become eligible for HIVDR testing. The Stanford HIV Drug Resistance Database is used to assess resistance to ART. HIVDR testing is not available in the public sector and hence patients need to access testing in the private sector at a prohibitive cost. Second-line treatment failure is confirmed by the presence of any level of resistance to atazanavir and lopinavir. Third-line ART for adults and children is composed of boosted darunavir, dolutegravir / raltegravir (raltegravir was used between 2015 and 2017 before dolutegravir availability) and sometimes two additional N(t)RTIs (based on the HIVDR test results).

Monitoring for ART treatment success was previously predominantly done using clinical events and routine CD4 measurements. Routine VL measurements became available in the public sector in 2015. ART regimen failure is defined as at least two VL results greater than 1000 copies /ml with good adherence to treatment in a patient who has been treated for at least six months. A VL test is mandatory to confirm diagnosis of second-line ART failure.

Data collection and analysis: Government clinics

We conducted a cross-sectional analysis using data from all four national third-line treatment centers. We included all patients who initiated third-line therapy between 01 January 2015 and 31 December 2018. We defined third-line therapy to be any treatment regimen that included darunavir and raltegravir or dolutegravir after documented PI based second-line failure. A data collection form was used to abstract individual patient level data (Demographic, laboratory, and ART history) from clinic files at Harare and Parirenyatwa Hospitals. Data from Bulawayo were accessed using the clinic's cohort report. The data collected included demographic variables, ART treatment history, viral load and CD4 count results.

Data collection and analysis: Newlands clinic

We analyzed data from NC in greater detail than data from the three hospitals because of completeness. Patient data were obtained from the clinic's electronic records. We described the population using proportions for categorical variables, medians and corresponding interquartile ranges (IQR) for continuous variables. Patients were followed from the date of third-line initiation until death, transfer, loss to follow up or data set closure, whichever occurred earlier.

We assessed the proportion of patients receiving third-line ART who achieved virological suppression (VL< 1000 copies/ml) after 24 and 48 weeks. Database was closed on 31 December 2018.

Ethical approval

This study was approved by the Medical Research Council of Zimbabwe, approval number: MRCZ/E/196. The ethics committee waived the requirement of informed consent as the study used routinely collected clinic data.

Results

Demographics

A total of 209 patients have ever received third-line ART through the national program as of 31 December 2018. Fig 1 highlights the number of patients from the four different third-line centers. The median age at commencement of third-line ART ranged from 38 years (IQR: 20–46.5) at Harare hospital to 48.7 years (IQR:43.7–50.7) at Parirenyatwa hospital. Table 1 summarizes the sociodemographic and clinical characteristics of patients who received third-line ART at the four clinics. Only NC had complete patient level data for baseline CD4 cell count and HIV viral load. We present results in two parts. First, we summarize the results of the third-line ART programme in the 3 government (public sector) linked clinics. We then present a more detailed assessment of the third-line program at the privately-run NC.

Public sector patients

Bulawayo clinic (Mpilo). Forty-nine patients had ever been initiated on third-line ART in Bulawayo and only 8/49 (16%) were initiated based on HIVDR test results. The HIVDR test results were not available for review. The rest were initiated after assessment by clinicians and consultations with senior doctors at the hospital. Among the 35 patients with available data on treatment duration, median duration of third line ART was 2.3 years (IQR: 0.6–3.4). Patients received second-line ART for a shorter duration (2.8 years, IQR: 1.9–4) compared to first-line ART (5.2 years). As of 31 December 2018, 7/49 (14%) had died, 3 were lost to follow up (LTFU) and 1 transferred out to South Africa.

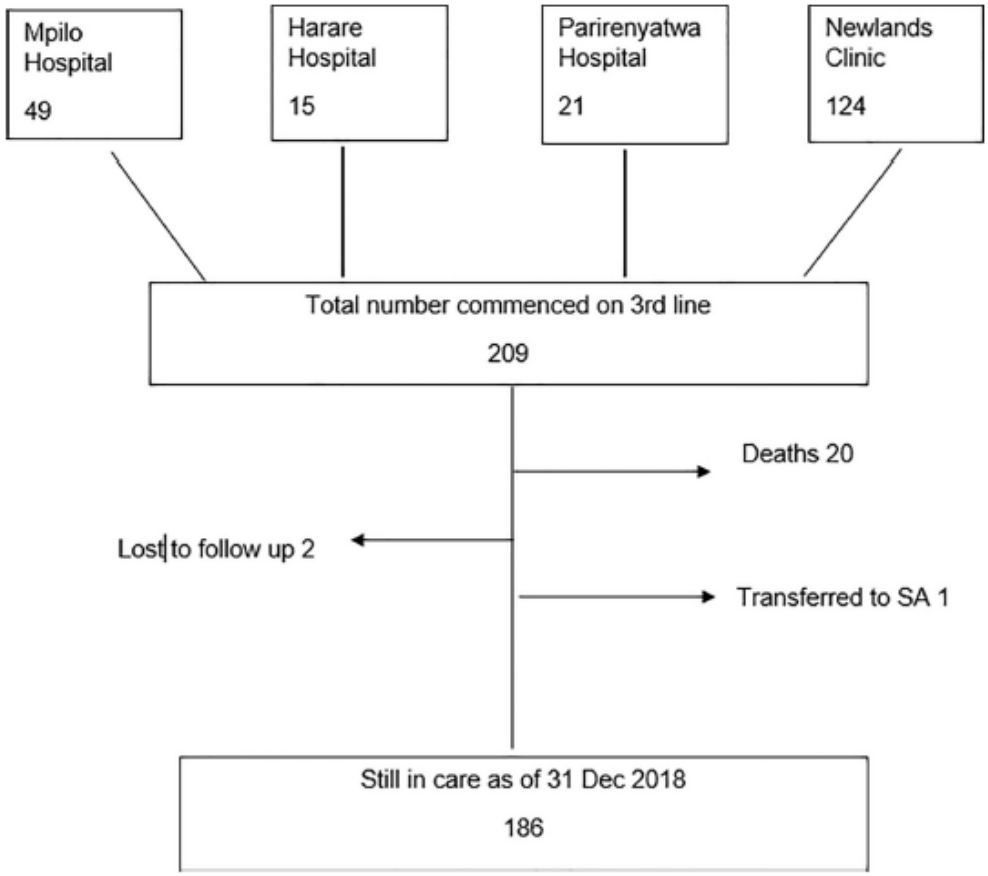

Fig 1. Patients commenced on third-line ART in Zimbabwe: 2015–2018.

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The total number of patients in care as at 31 December 2018 was 38, and 33 of them had received third-line ART for more than 6 months. Of the 33 on treatment for more than 6 months, 25/33 (76%) had a documented VL result during the last 12 months and 22/25 (88%)

Table 1. Sociodemographic and clinical characteristics of patients receiving third-line antiretroviral therapy in Zimbabwe.

Parameter: Median (IQR)	Newlands (n = 124)	Parirenyatwa (n = 21)	Harare central hospital (n = 15)	Bulawayo (Mpilo) (n = 35)**
Baseline age (yrs.)	42.1 (27.6–48.5)	48.7 (43.7–50.7)	38 (20–46.5)	44 (35.1–53.3)
Duration of first-line ART (yrs.)	4.5 (2.8–6.4)	3.3 (2.2–7)	3.5 (2.1–4.6)	5.2 (2.5–7.7)*
Duration of second-line ART (yrs.)	3.3 (2.2–6.4)	3.1 (2.8–6.2)	4.4 (2.4–5)	2.8 (1.9–4)*
Duration of third- line ART (yrs.)	1.4 (0.6–2.8)	1.3 (0.7–1.7)	1 (0.6–1.9)	2.3 (0.6–3.4)
Baseline CD4 cell count (cells/mm3)	116 (27–245)	-	-	-
Baseline viral load (copies/ml, IQR)	78 845 (17 346–256 329)	-	-	-
Number of women (%)	61 (49)	7 (33.3)	7 (46.7)	18 (51.4)

*Only 21 patients had complete data: ART = Antiretroviral therapy

** Data presented for patients with available clinic records

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had a VL below 1000 copies/ml. Two patients had low level viremia of between 200 and 1000 copies/ml.

Harare clinics. Thirty-six patients (22 males and 14 females) were commenced on third-line ART from the two government treatment centers in Harare (15 from Harare hospital and 21 from Parirenyatwa hospital) as of 31 December 2018. Only five patients had available documented HIVDR results and all 5 patients had triple class (PI, NNRTI and NRTI) resistant HIV. The median duration of third-line ART was 1.3 years (IQR: 0.7–1.7) at Parirenyatwa hospital and 1 year (IQR: 0.6–1.9) at Harare Hospital. Thirty-four patients had been receiving routine VL monitoring, and for 31/34 (91%) patients their most recent VL was < 1000 copies/ml after at least six months of third-line ART.

Newlands Clinic third-line program

Baseline characteristics (third-line ART commencement). Since inception of the program at NC, 124 patients (49% female) had started third-line ART. Only 9/124 (7.3%) patients commenced first-line ART at NC and the remaining 115/124 (92.7%) were transfers from other HIV treatment centers from across the nation for either first, second- or third-line ART. The median age at third-line commencement was 42.1 years (IQR: 27.6–48.5). The youngest and oldest ages at third-line commencement were 7.7 years and 71 years respectively. Median CD4 cell count and VL at third-line commencement were 116 cells/mm^3 (IQR:27–245) and 78,845 copies/ml (IQR: 17,436–256,329) respectively. Patients had received first- and second-line ART for a median duration of 4.5 years (IQR: 2.8–6.4) and 3.3 years (IQR: 2.2–5.4) respectively.

HIV drug resistance. A total of 89 patients (72%) had documented HIV genotyping results and 65/89 (73%) had at least one major PI resistance associated mutation (RAM). The most prevalent PI RAM was V82A/L/M/C/F which was present in 42.7% of the 89 patients. The commonest NNRTI RAM was G190A/S/E which was prevalent in 32.6% of the 89 patients and the M184V mutation was the commonest NRTI RAM present in 91% of the 89 patients. Fig 2 displays the frequency of the different RAMs. Of the patients commenced on third-line ART, 24 did not have PI resistance mutations. Unfortunately, these patients were commenced on treatment prior to receiving the HIVDR tests results due to delays. After results were received, clinicians decided to continue with the third-line regimen despite the lack of PI mutations.

Third-line treatment outcomes. As at 31 December 2018, 88% (109/124) of patients who commenced third line ART were still in care, 10.5% (13) had died and 1.5% (2) were transferred to other treatment centers. Median duration of third-line ART was 1.4 years (IQR: 0.6–2.8). Among the 111 patients who had received third-line ART for at least 6 months, 105 (95%) had a achieved a VL of < 1000 copies/ml [75% (83) had a VL <50 copies/ml, 16% (18) had a VL of 51–200 copies/ml, 4.5% (5) had a VL of 201–1000 copies/ml] Among the 51 patients who had week 48 VL results, 48 (94%) patients had a VL < 1000 copies/ml. One patient, a 19-year-old adolescent girl, failed third-line ART and HIVDR testing confirmed high level resistance to all protease inhibitors, reverse transcriptase inhibitors and integrase inhibitors including dolutegravir. The patient was switched to a holding regimen of zidovudine, lamivudine and abacavir and details have been published as a case report [15].

Mortality. At the time of death, the 13 deceased patients (7 males and 6 females) patients had received third-line ART for a median duration of 7.7 weeks (IQR: 3–23.2). The causes of death were as follows: 3 patients died of renal failure and 3 patients died of Non-Hodgkin's Lymphoma. Other causes of death were (one patient per cause): liver failure, anemia, gastroenteritis, milliary tuberculosis, chronic pancreatitis (alcohol induced), unknown, and deep vein

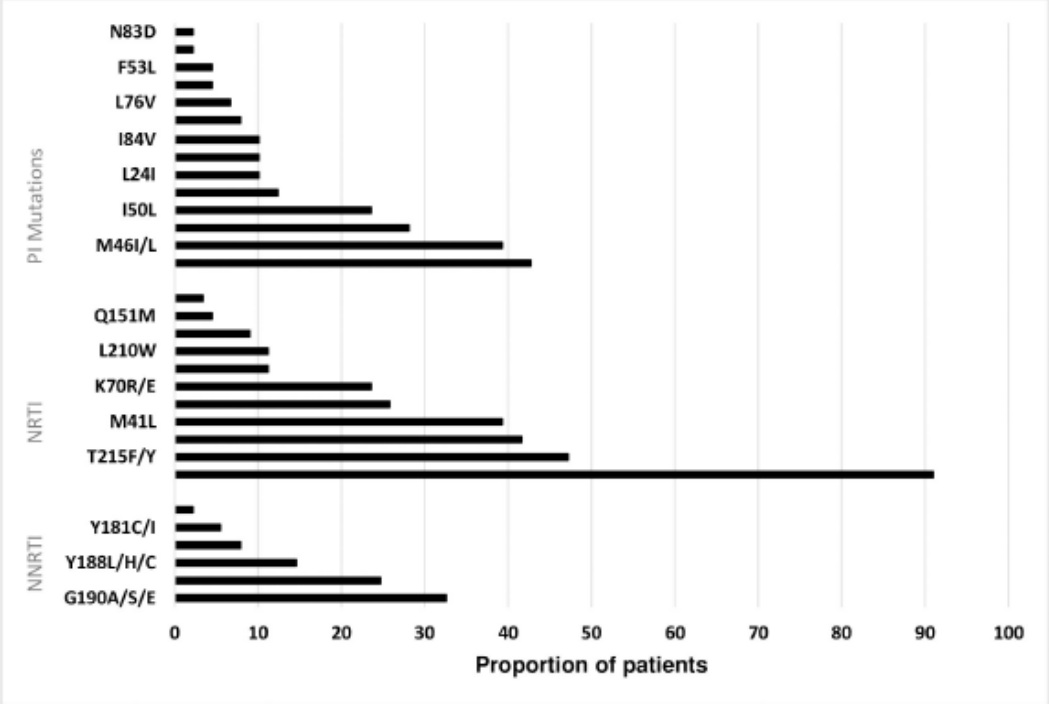

Fig 2. Distribution of HIV drug resistance mutations at Newlands Clinic.

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thrombosis. At the time of death three patients had achieved virological suppression on third-line ART.

Discussion

We present the findings of the outcomes of the Zimbabwe national public sector third-line ART cohort. These findings are important because very few countries in sub-Saharan Africa provide third-line ART in public sector programs, hence there is scarcity of data on third-line ART in these settings. We show that there is poor access to HIVDR testing among patients failing second-line ART in the government led treatment centers. Among patients with access to HIVDR testing, there was a high prevalence of RAMs affecting PIs, NNRTIs and NRTI drug classes. Patients receiving third-line ART at NC have high virological suppression rates, with 94.5% being suppressed to below 1000 copies per milliliter. Despite the lack of complete patient level data, good treatment outcomes were achieved among patients receiving third-line in the public sector clinics.

The poor access to HIVDR testing for patients failing PI-based second-line ART in the government clinics is of great concern. While Newlands Clinic had higher rates of HIVDR testing, rates were still not 100%, which indicates a more universal issue regarding access to HIVDR testing, rather than an issue that is isolated to the public sector.

Previous studies of second-line ART outcomes in adults in SSA found that acquired resistance associated with protease inhibitors was infrequent and treatment failure was mainly due to poor adherence [7,8,10,16–18]. Clinical management of second-line failures is sub-optimal

in the absence of resistance testing to identify people who harbor clinically relevant RAMs and need an optimized third-line regimen. Poor access to HIVDR testing and VL monitoring in Zimbabwe presents a very important challenge for the provision of optimum care for HIV infected patients. A simulation and cost-effective analysis projected that HIVDR testing and third-line ART would increase survival and be cost-effective in resource-limited settings compared to a public health approach of using a standard third line regimen [19,20]. Our findings of high prevalence of triple class resistance after second line ART failure in the NC cohort are consistent with previous studies [21,22]. Delays in switching patients due to poor availability of routine VL monitoring may explain the high prevalence of triple class resistance.

Our data demonstrated the effectiveness of third-line ART in a programme where the predominant subtype was C. Our findings are in line with other studies from the region that demonstrated good virological suppression rates among patients receiving third-line ART [11,12,23]. Findings from studies in resource limited settings have demonstrated that virologic suppression is a realistic endpoint for most treatment-experienced patients who begin a darunavir-based third-line therapy outside the controlled conditions of a randomized trial, at routine care settings [24–26]. NC has a very intense program to help prepare patients for third-line ART which includes a six-week enhanced adherence support program. The program focusses on identifying and addressing barriers to optimum ART adherence. Furthermore, NC also assist the very poor patients with bus fare and food to help keeping these patients engaged in care. Among the patients who died, the median duration of third-line ART was only 7.7 weeks. Early diagnosis of second-line ART failure and subsequent switch to third-line ART may help reduce mortality among these patients. The NC model of care can be replicated in the public sector clinics, however, this will require greater investment of resources in training of health workers and equipping clinics with electronic monitoring systems. We do acknowledge that that targeted retention programs (vouchers for food and transport) and electronic medical record systems at NC would have significant costs, which may not be feasible in the public sector.

The Zimbabwe national third-line ART program is facing several challenges. In our view the challenges are mainly because the program is centralized to the two largest cities in the country. This increases costs for third-line patients who are seen more frequently compared to those on first- and second-line treatment. HIVDR testing is not available in the public sector and hence patients need to access this test from the private sector at high costs unaffordable to most public health patients. Due to this prohibitive cost, management of patients is negatively affected as some end up being switched on clinical grounds without genotypic testing which overestimates those eligible and has cost implications to the program. Low VL testing coverage as reported by the national ART coordinator (44% at the end of 2018) is a challenge and impacts negatively on patient monitoring. We anticipate similar challenges in other national ART programs in SSA as countries scale up the provision of third-line ART. There is need to decentralize the provision of third-line ART to the provinces and districts to increase accessibility. Lessons can be learned from the South African third-line program were decentralization has been achieved through the aid of a well-coordinated national third-line committee [12].

The main limitations of our study are the relatively small sample size, the short duration of follow up and the missing individual patient data from the three government linked clinics. The lack of complete data regarding the number of patients failing second-line ART makes it difficult to contextualize the need for third-line ART in Zimbabwe. However, the lack of complete data is an important finding that highlights the need for the national program to invest in better and more user-friendly electronic monitoring systems. Regardless of these limitations, we find these data useful in helping national programs in the region improve care of patients post second-line ART.

Conclusions

Our findings highlight the need for national ART programs especially those in resource limited settings to strengthen access to HIVDR testing for patients failing second-line ART. HIVDR testing is essential for the identification of patients who have acquired clinically relevant HIV resistance associated mutations, furthermore, it helps to optimize third-line regimens. This study has also demonstrated that with comprehensive care, patients who have failed on PI based second-line ART can achieve high rates of virological suppression on third-line ART. As more patients are likely to fail second-line ART, national programs must ensure that third-line ART is available through decentralization of care. Healthcare workers must be trained and equipped to identify and manage patients failing second-line therapy early.

Supporting information

S1 Dataset.
(DTA)

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Author Contributions

Conceptualization: Cleophas Chimbetete, Olivia Keiser.

Data curation: Cleophas Chimbetete.

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A case report of untreatable HIV infection in Harare, Zimbabwe

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Introduction: Zimbabwe, like other resource limited countries, manages HIV infection using the public health approach with standard antiretroviral therapy (ART) regimens for first, second and third-line treatment. Third-line ART is the last available treatment option and is based on dolutegravir and darunavir use after HIV drug resistance testing.

Patient Presentation: We report here a 17-year-old patient on dolutegravir (DTG) and Darunavir based third-line antiretroviral therapy (ART) previously exposed to raltegravir who develops multidrug resistance HIV to the four ART classes available in Zimbabwe.

Management and Outcome: A trophism assay revealed that patient has CXCR4 trophic virus and hence will not benefit from Maraviroc. Patient is currently stable and receiving a holding regimen of abacavir, lamivudine and lamivudine.

Conclusion: This is the first documented case of multiclass resistance to the four available ART classes in Zimbabwe. The development and transmission of multiclass HIV drug resistance in resource limited settings has potential to undo the gains of national ART programs. There is need to ensure optimum adherence to ART even in the era of DTG.

Keyowrds: Dolutegravir; Resistance; Untreatable HIV; Zimbabwe; ART programmes.

Background

Widespread availability of antiretroviral therapy (ART) has transformed a positive HIV diagnosis from being a death sentence into a chronic manageable disease. To date, no cure exists for HIV, and hence patients must remain on effective ART for the rest of their lives, that makes the development of drug resistance a major public health concern. Sustained viral suppression is of paramount importance if drug resistance is to be prevented. Strategies to ensure optimal adherence to ART are, therefore, an important component of HIV care and treatment. Antiretroviral therapy resistance limits further treatment options, increases treatment programme costs and drug resistance may even be transmitted to others.[1] The rising prevalence of HIV drug resistance poses a great threat to the HIV response and has the potential to drive increase in mortality and HIV incidence.[2] Several risk factors for the development of HIV drug resistance among patients on ART have been identified.[2]

HIV treatment in Zimbabwe is based on a public health approach using standard national treatment guidelines.[4] Treatment guidelines have periodically changed and are guided by the World Health Organization (WHO). In 2015, Zimbabwe introduced third-line ART in the national programme. Patients failing second-line ART are referred for specialist assessment that includes viral load (VL) and genotype testing prior to recommending third-line medicines. Adherence needs to be reinforced at all times.[4]

We report the first case of documented four-class HIV drug resistance in Zimbabwe that highlights the possibility of third-line ART failure and transmission of untreatable HIV in resource-limited settings.

Case report

We report the case of an adolescent girl born in July 2000. She tested positive for HIV infection in 2009 and was enrolled into care at Newlands Clinic on 30 July 2009. She is the last born in a family of three children, a paternal orphan and stays with her mother. She was vertically infected, and her mother is accessing ART at the same treatment centre. Both her siblings are HIV negative. She commenced first-line ART on 28 August 2009. Table 1 summarises ART regimens received over time and the reasons for regimen changes.

Monitoring for ART treatment success was done clinically and immunologically since the initiation of treatment. Routine VL monitoring was added in January 2014. Figure 1 highlights the patient's CD4, VL and ART regimens over time.

HIV drug resistance testing and third-line response

A genotypic resistance test was performed on 31 March 2015 after second-line ART failure. Results of the test were interpreted using the Stanford HIV drug resistance guide. We found four major protease inhibitor (PI) resistance-associated mutations (RAMs), that is, M46I, I54V, L76V and V82A. The PI RAMs conferred high-level resistance to atazanavir (ATV), lopinavir, indinavir and saquinavir.

There were three nucleoside reverse transcriptase (NRTI) RAMs, that is, M41L, M184V and T215F, and three non-NRTI RAMs, that is, A98G, K103N and E138A. The RAMs conferred intermediate resistance to abacavir, zidovudine, stavudine, didanosine and rilpivirine. There was high-level resistance to emtricitabine, lamivudine, efavirenz and nevirapine. The virus had low-level resistance to tenofovir and etravirine. Table 2 summarises results of the resistance tests conducted during the course of patient management.

TABLE 1: Antiretroviral therapy history by regimen.

ART regimen	Start date	End date	Reason for switch
d4T/3TC/NVP	28/08/2009	30/07/2010	Guideline change
AZT/3TC/NVP	30/07/2010	10/04/2012	Treatment failure
LPV/r/AZT/3TC	10/04/2012	20/01/2015	Guideline change
ATV/r/3TC/ABC	20/01/2015	12/08/2015	Treatment failure
RAL/DRV/r/3TC	12/08/2015	28/07/2016	Clinic decision
DTG/DRV/r/3TC	28/07/2016	21/03/2017	Poor adherence
3TC Monotherapy	21/03/2017	23/01/2018	Change to effective regimen
DTG/DRV/r/3TC	23/01/2018	12/11/2018	Changed to holding regimen
ABC/3TC/AZT	12/11/2018	Current	-

ART, antiretroviral therapy; d4T, stavudine; AZT, zidovudine; 3TC, lamivudine; NVP, nevirapine; ABC, abacavir; r, ritonavir; DRV, darunavir; ATV, atazanavir; LPV, lopinavir; RAL, raltegravir.

She was started on third-line ART in August 2015. She has had challenges with treatment adherence because of the high pill burden, and received 3TC monotherapy as a holding therapy from March 2017 (VL was 255 397 copies/mL) to January 2018. She was treated for pulmonary tuberculosis (TB) from 08 August 2017 to 23 January 2018. The TB diagnosis was made based on loss of weight and suggestive chest X-ray findings. She improved clinically on TB treatment, and after completing 6 months of therapy, she recommenced third-line therapy with ritonavir-boosted darunavir, lamivudine and dolutegravir (DTG). She came daily to the clinic for a nurse to observe her and to take third-line medicines for 16 weeks, but her VL remained very high.

An integrase strand transfer inhibitor (INSTI) resistance test was then performed on 14 June 2018. Results showed three integrase inhibitor major RAMs, that is, E138K, G140A and Q148R. The RAMs conferred high-level resistance to DTG, raltegravir (RAL) and elvitegravir (ELV). Trophism assay was performed, and results showed that unfortunately the patient is CXCR4 trophic and hence maraviroc is unlikely to work. The recently approved post-attachment inhibitor, ibalizumab, is not available in the country. She was commenced on a holding regimen of ABC, 3TC and AZT, and her latest VL done on 12 November 2018 was 771 334 copies/mL. Her mother is virologically suppressed on a second-line ART regimen of ATV or ritonavir, AZT and 3TC.

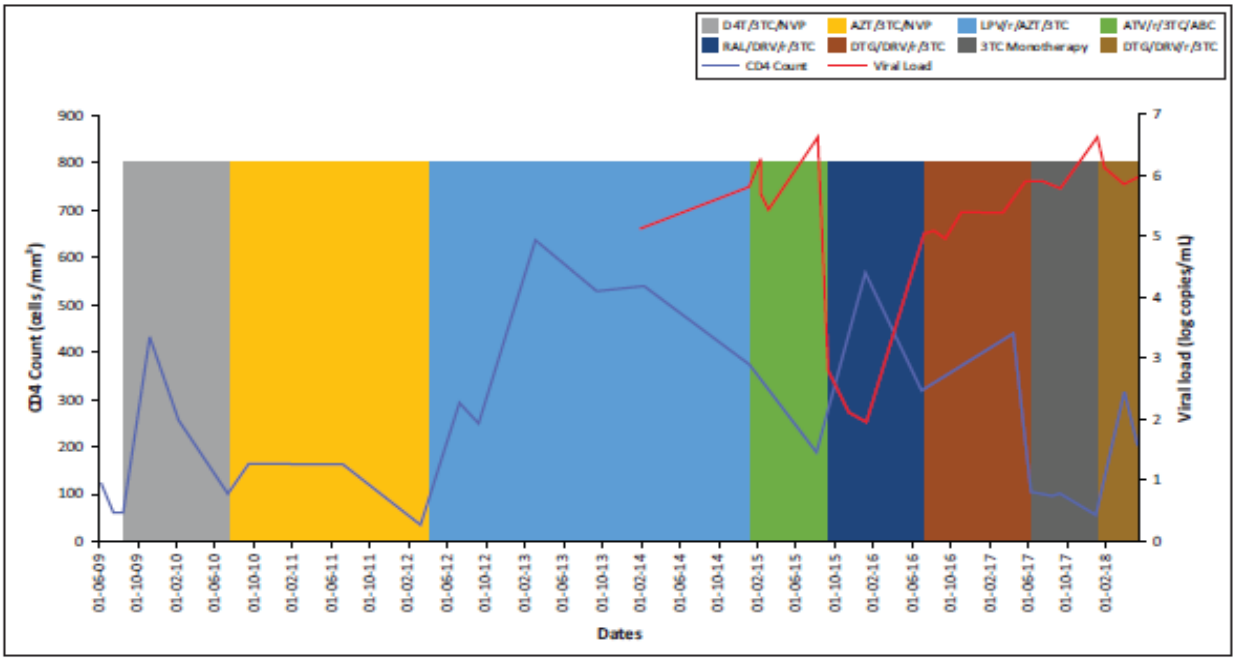

FIGURE 1: CD4 count, viral load and antiretroviral therapy regimens over time.

TABLE 2: HIV drug resistance test results.

	2015		2016	
Medicines	**Mutations**	**Description of resistance**	**Mutations**	**Description of resistance**
NRTI	*M41L, M184V, T215F*		*M41L, M184V, T215F*	
Zidovudine		Intermediate		Intermediate
Lamivudine		High level		High level
Abacavir		Intermediate		Intermediate
Emtricitabine		High level		High level
Tenofovir		Low level		Susceptible
NNRTI	*A98G, K103N, E138A*		*A98G, K103N, E138A*	
Nevirapine		High level		High level
Efavirenz		High level		High level
Rilpivirine		Intermediate		Intermediate
Etravirine		Low level		Low level
PI	*M46I, I54V, V82A*		*M46I, I54V, V82A, L76V*	
Lopinavir		High level		High level
Atazanavir		High level		High level
Darunavir		Susceptible		Intermediate
INSTI†			*E138K, G140A, Q148R*	
Elvitegravir				High-level resistance
Raltegravir				High-level resistance
Darunavir				High-level resistance

NRTI, nucleoside reverse transcriptase inhibitors; NNRTI, non-nucleoside reverse transcriptase inhibitors; INSTI, integrase strand transfer inhibitor.
†, Test done on 14 June 2018.

Ethical consideration

Analysis of routine clinical data is approved by the Medical Research Council of Zimbabwe as part of a larger study, International Epidemiological Databases to Evaluate AIDs (IeDEA Collaboration) (approval no. MRCZ/A/1336). Verbal assent from adolescent and written informed consent from parent were obtained.

Discussion

To our knowledge, this is the first report of a patient with a virus that has developed multi-class drug resistance to all four standard classes of ART, including INSTIs, in Zimbabwe. This patient has HIV with high-level resistance to DTG after previous exposure to RAL. Recently, a case of multi-drug resistant HIV, including resistance to INSTIs, was reported from Botswana[5] and a similar case was reported earlier in South Africa.[6] Multi-drug resistant HIV could have developed because of a variety of factors, including poor adherence to ART and inadequate psychosocial support – issues which are frequently encountered among adolescents living with HIV.[7] In this case, poor adherence was mainly because of poor family support and lack of motivation for ART when the patient felt clinically well. Poor adherence to previous ART regimens could have led to exposure to DTG monotherapy. Previous studies have shown that monotherapy with DTG has a high rate of resistance selection in the integrase gene through different pathways in case of virologic failure.[8]

Integrase strand transfer inhibitors are one of the newest class of antiretroviral drugs to be approved for HIV treatment and act by inhibiting the essential HIV protein integrase from inserting the viral DNA genome into the host cell's chromatin. Raltegravir and EVG have been successful in clinical settings, but have low genetic barriers to resistance. Dolutegravir is known to have a very high genetic barrier to resistance and retains activity against RAL- and EVG-resistant viruses.[9,10] Zimbabwe has not yet adopted the use of DTG as part of the preferred first-line ART regimens.

Conclusion

This is the first case of recorded four-class HIV drug resistance in Zimbabwe. This adolescent girl cannot be effectively treated with any of the currently available ART regimens in Zimbabwe. Prevention measures such as family planning intervention and safe sex counselling are being taken to minimise the risk of transmission of this multi-class resistant virus.

This case emphasises the need for health workers to continue providing adherence counselling and support for patients who are on ART. Transmission of four-class-resistant HIV is a potential public health disaster.

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Competing interests

The authors declare that they have no financial or personal relationship(s) that may have inappropriately influenced them in writing this article.

Authors' contributions

L.C. and M.P. are the physicians looking after the patient. C.C. and L.C. prepared the first draft of the case report. All authors read and approved the final manuscript.

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Conclusion and Perspectives

Poor adherence and lack of protease inhibitor mutations

This thesis details the current state of the Zimbabwe national third-line ART program. It identifies major gaps and lessons that are crucial for the optimum management of HIV infected patients after second-line ART failure. Consequently, a number of recommendations have been put forward to the Ministry of Health and Child Care to assist in strengthening the national third-line program. With the support of different partners, implementation of these recommendations has begun.

One of the key findings is the lack of protease inhibitor associated resistance in more than 50% of the patients failing protease inhibitor based second-line ART despite intensive adherence counselling and support. A number of previous studies from resource limited settings like Zimbabwe have also reported low rates of protease resistance after second-line failure attributing this finding to poor medication adherence [29,31,48]. Given the higher cost of third-line ART, it becomes imperative for national ART programs to develop clear guidelines and protocols that help healthcare workers (HCW) identify and support patients struggling with medication adherence for whatever reason. HCW need to be afforded adequate time with patients to enable them to explore and identify potential barriers to medication adherence. Some of these barriers may be outside the patient's control e.g. stock outs of essential medicines, lack of food, mental health challenges and medicine toxicities. Furthermore, there is need to employ a multi-disciplinary team approach in the care of patients failing second-line ART. The role of social workers, community peer supporters, nurses and clinical psychologists cannot be over emphasized. A comprehensive approach involving the patient, health workers and the community is an effective strategy to strengthen adherence among treatment experienced patients who have received multiple ART regimens [49]. As more patients fail second-line ART in resource limited

settings and the need for more complex third-line regimens increases, there is need to involve the community more. Various studies have demonstrated that community-based programs address social barriers to care, promote retention of patients in care and adherence to ART. [49,50]. In order to reach global targets of ending HIV and AIDS by 2030, it will be crucial to include a combination of clinic based and community-based strategies that facilitate universal access to all ART regimens [51].

Zimbabwe has adopted the differentiated service delivery model (DSD) approach to HIV management. The DSD model is a patient-centered approach to HIV prevention, care and treatment [52]. This model has moved away from a one-size-fits all model of HIV care. The strategy adapts HIV services for diverse groups of people living with HIV while maintaining the principles of public health approach. Through this model health workers are able to spend more time with patients failing second-line ART and those on third-line ART increasing efficiency and improving quality of care. Expectations are that the DSD models will help improve the quality of care offered to patients failing second-line ART in Zimbabwe.

ART programs must address the many social barriers to care, promote retention of patients in care and adherence to ART. Failure to address medication adherence challenges will lead to unnecessary switches to expensive third line ART which will in turn fail due to poor adherence as well. Our findings have confirmed that poor adherence is the key challenge to address in patients failing protease inhibitor based second-line ART and furthermore, we have shown that this is a manageable problem.

Poor access to HIV drug resistance testing

Poor access to HIVDR testing in Zimbabwe is a great cause of concern. Clinical management of second-line ART failure in the absence of resistance testing to identify people who harbor clinically relevant resistance mutations and need an optimized third-line regimen is suboptimal. Patients are forced to continue on a failing PI based second-line ART while awaiting resistance testing which in turn leads to development of cross resistance to the PI darunavir (third-line medicine). In several low- and middle-income countries like South Africa, Botswana, Brazil and Uganda, routine resistance testing is now being done in patients failing second-line ART. There have been mixed reports on the cost effectiveness of HIV drug resistant testing after first line ART failure [40,41,53,54]. However, it is clear that it is cost effective to do HIV genotyping before switching to third-line ART [40]. The Zimbabwe national ART program through the support of partners is in the process of strengthening laboratory capacity to enable provision of affordable HIV drug resistance testing. It is anticipated that resistance testing will be available in Zimbabwe through the national program in mid-2020.

The World Health Organization has set the ambitious target of at least 90% of HIV infected people on ART achieving virological suppression by the year 2020. Sadly, the potential positive impact of the achieved widespread availability of ART is under significant threat from the growing burden of HIVDR in the region. Obtaining quality data on HIVDR from periodic surveys: expanding the coverage of routine viral load and HIVDR testing in Zimbabwe is difficult due to lack of access to HIVDR testing. There is an urgent need to ensure that governance and enabling mechanisms (advocacy, country ownership, coordinated action and sustainable funding) are in place to support the country's action on HIVDR. Strong political engagement and leadership are essential to enable Zimbabwe to implement a participatory national strategy which ensures accessibility to HIVDR testing.

Together with increased access to HIV viral load monitoring in resource limited countries, feasible and affordable HIVDR testing technologies are required to help maximize the rationale use of available ART medicines. There are several barriers to access of HIVDR testing in resource limited countries such as Zimbabwe. These barriers include need for high capital investment and test costs, limited molecular laboratory infrastructure, lack of appropriately trained staff and need to maintain a complex cold-chain sample logistics.

There are promising low-cost technologies for HIVDR testing in low- and medium-income countries (LMICs). Genotypic resistance test (GRT) assays can be classified as point mutation assays (PMAs) and sequencing based assays. The sequencing-based assays are classified into Sanger and next-generation sequencing (NGS). The PMAs are low cost and only identify specific sequence changes on the HIV genome. PMAs have high sensitivity for minor mutation variants and they can be used in a point-of-care test platform [55]. Inexpensive near point-of-care (POC) GRT would be useful in LMICs where the resources, capacity, and infrastructure to perform standard GRT are limited. However, the PMA technology has a significant disadvantage in that there is HIV-1 sequence variability at each drug resistant mutation target, meaning that multiple experiments are needed per each target site. The increased number of experiments increases the costs of PMAs [56]. Implementation and scale up of low cost HIVDR testing in Africa has the potential to advance ART decision-making both for individual patients and policy formulation. Decentralization of HIVDR testing can be achieved through the improvements of existing POC technologies.

Absence of HIVDR testing should not be an obstacle to the prescription of third-line ART. Recently, researchers in West Africa demonstrated that a standardized algorithm based on intensive adherence counselling and repeated viral load monitoring is a reasonable strategy for the management of patients failing second-line ART [57]. Alternative approaches that evaluate

and rank a set of suitable treatment regimens to select the most suitable ART regiments for patients failing treatment exist. Machine learning methods, which make use of computational models to provide predictions of virological response to ART on the basis of large databases of ART-treated patients may be used [56].

Decentralizing of third-line ART in Zimbabwe

Third-line ART is currently only available at four clinics in Zimbabwe. These clinics are situated in the two biggest cities of Harare and Bulawayo. Consequently, access to third-line treatment among patients from outside these two major cities has been a huge challenge. As illustrated in the map below, Zimbabwe is much bigger than these two cities. Furthermore, our assessment showed that the management of patients failing second line is not uniform across the four sites and needs to be standardized.

Fig 1: Map of Zimbabwe

As more patients fail second-line ART, and the harsh economic environment, an urgent need has arisen to decentralize the provision of third-line ART across the country's ten provinces. Following the results of the assessment of the national third-line program, the Ministry of Health and Child Care (MOHCC) through the AIDS and TB unit has been engaged to initiate the process of decentralizing third-line ART. A committee, which I co-chair, has been established to spearhead the process. The overall objective of the third-line committee is to provide technical support to the MOHCC's national HIV treatment program on standardization of the management of patients failing second-line ART and to facilitate decentralization of third-line ART. Third-line

ART will be decentralized initially to sites that have resident specialist Physicians and Pediatricians. Two sites, Mutare and Gweru provincial hospitals have been identified for the first phase of the decentralization. Additional sites will be identified, and this will depend on need and resource availability. Newlands Clinic (NC) will provide training and mentorship to both Mutare and Gweru provincial hospitals. Further decentralization of third-line ART across the nation will be spearheaded by a virtual third-line committee.

Functions of the national third-line committee

Clinicians across the nation will email detailed case summaries of patients suspected of failing second-line ART to the committee's coordinator. The case summary includes demographic data; contact details of the referring clinician and facility; history of previous ART regimens and reasons for stopping or changing ART; weight and body mass index; adherence history; concomitant medications (such as tuberculosis treatment); basic laboratory results (creatinine and creatinine clearance; hemoglobin; and hepatitis B surface antigen) as well as recent CD4+ counts and viral load; and the genotype result. This information is collected and collated centrally by the third-line coordinator. The coordinator then circulates all the collated information through email with the resistance genotype and Stanford scores to the third line committee. The committee assesses eligibility for third-line ART and makes a regimen recommendation for each individual case based on information received. Once the regimen is agreed upon, the medications are released on a named-patient basis to the facility attended by the patient. The MOHCC will require the support of funding partners to enable efficient decentralization of third-line ART in Zimbabwe. Partners will be required to support the following:

1. Training and mentorship of new third-line sites
2. Availability of HIV resistance testing which is mandatory before a diagnosis of second-line ART failure can be made

3. Uninterrupted availability of third-line medicines at all sites
4. Effective monitoring of patients on third-line ART (access to essential laboratory tests e.g. HIV viral loads at all sites)
5. Monitoring and evaluation i.e. national third-line outcomes
6. Activities of the national third-line committee

The committee shall, on a quarterly basis, share reports with the MOHCC's national HIV treatment coordinator. The report will detail all cases reviewed and assessed for third-line eligibility and track treatment outcomes across the continuum of care. Reports will also cover progress achieved on the decentralization of third-line management, emerging concerns being noted and recommendations for program improvement.

ART Treatment after third-line failure

As more patients access third-line ART in LMICs, program managers must begin to plan for the possibility of third line failure. We reported the first case of documented four-class HIV drug resistance in Zimbabwe that highlighted the possibility of third-line ART failure and transmission of untreatable HIV in resource-limited settings [36]. This is a case of an adolescent girl who developed confirmed HIV drug resistance to all available ART medicines in Zimbabwe. Multi-drug resistant HIV could have developed because of a variety of factors, including poor adherence to ART and inadequate psychosocial support – issues which are frequently encountered among adolescents living with HIV [58]. Similar cases of multi-drug resistant HIV have been reported in Southern Africa [59,60]. These cases highlight the need for continued monitoring of HIV-1 VL and the development of robust HIV drug resistance surveillance systems for failing and highly treatment-experienced patients.

Resources must be continuously availed to ensure access to new ART classes like co-receptor inhibitors and maturation inhibitors for heavily treatment experienced patients in LMICs. Long-

acting therapies that—unlike current antiretrovirals, which require daily dosing—could be taken only once a week, once a month, or even less often must be developed. Such long-acting therapies might be able to improve adherence especially among adolescents and young people and might also be less toxic and more cost effective. Currently new types of anti-HIV agents are under study, these are long-acting drugs, broadly neutralizing antibodies, and therapeutic vaccines.

With all the innovative new agents being developed, it is possible to maintain virological suppression in HIV positive patients living in LMICs after failure of third-line ART.

Introduction of dolutegravir based first line ART

The integrase inhibitor, dolutegravir (DTG), has a major role in ART regimens in sub-Saharan Africa. This is because of its high potency, low cost, excellent tolerability and high genetic barrier to resistance. Countries have begun transitioning all patients on first-line ART from an efavirenz based regimen to a DTG based regimen. The transitioning will help address the challenges of rising NNRTI resistance and improve treatment outcomes, however, it presents new challenges.

The need for viral load and resistance testing might be increased with roll out of combined tenofovir, lamivudine, and DTG. There is risk of DTG functional monotherapy if transitioning without viral load testing is done among patients who have already acquired resistance to the NRTI backbone. DTG maintenance monotherapy studies have reported high virological failure with acquisition of integrase inhibitor resistance mutations [61,62]. The use of DTG as part of first- and second-line regimes may affect third line regimens in future.

Appendix

Enhanced Adherence Counselling Group Intervention

Introduction

Adherence is one of the most important aspects of living with HIV. However, it is also probably one of the most difficult. Poor Adherence leads to poor health outcomes and increased health care costs. This intervention is designed to help participants realise that whilst HIV may currently not be curable, Antiretrovirals present a significant positive in HIV management and wellbeing.

Goals

- To provide a supportive space where patients can talk about their experience of HIV, ART & Adherence.
- To find out the enablers of good treatment adherence and reinforce and strengthen these factors
- To unravel and understand at a deeper level issues that hinder patients from taking their medication.
- Ultimate goal to try to address issues of non-adherence at the different levels with the hope of empowerment & improving a patient's adherence.

Target: Patients who have high viral loads, failing on ART treatment

Length: 6 1.5 hour sessions

Size: 8 to 10 participants

Session One

Materials

- Blank index cards/paper
- Copies for each participant of PHQ-9 (Shona/English)
- Consent forms for each participant

Activities

I. Introduction (15 minutes)

- The purpose of the therapy group
- Consent forms explained

Have group members tell their names and say a little of themselves. *Examples*

- Things about them that they think is important
- Main interests
- Family, where they grew up

Establish Group etiquette and rules

- Come on time
- Make a commitment to come every week
- Be supportive of each other
- Respect and give each person a chance to talk
- Confidentiality: do not discuss personal things shared in the group with people outside of the group. However, you can discuss what you are learning about adherence with others.
- Tell us when you are unhappy; bring your concerns.

II. Icebreaker (10 mins).

Purpose: allows group members to become more familiar with each other in a non-threatening manner, creating a good atmosphere for learning and participation. The ice breaker facilitates members becoming comfortable participating in group activities.

III. Conceptualising HIV, ART & Adherence

- Thoughts that come to mind when you think of HIV
- Thoughts that come to mind when you think of HAART
- Thoughts that come to mind when you think of Adherence

Purpose: gives insight into meanings attached to one's status, patients' understanding of health and what adherence is.

IV. Group discussion of points raised above
V. Hopes and fears of the group process exercise
VI. PHQ-9

Session Two

Materials

- HIV & ART quiz questionnaire
- Large sheets of paper

Activities

I. Recap of last week (15 minutes)

A discussion around topics discussed in the previous week. Group members can be encouraged to share their thoughts and feelings about coming to group therapy and any changes if any have been noted. A review of the previous week also allows you to assess what stood out for participants as well as refresh their memory.

II. Adherence Facts (Yes or No activity) (45minutes)

Purpose: to assess knowledge members have regarding adherence, medication and HIV. The activity also creates a forum for peer-to-peer information sharing and advice
Allows one to teach members critical information in a non-traditional format.

- Write YES and NO on the large sheets of paper
- Members stand in the middle of the room while you read questions
- Members have 30 seconds to think about their answer without consulting
- After 30 seconds shout GO and members should move either to the Yes or No paper
- Ask members why they have chosen a particular answer before giving the correct answer and take note of a possible debate
- It is also helpful to ask members at the end if they have any yes/no questions of their own

III. **Ask members to each write down topics/questions** they would like addressed that impacts on their adherence (20 minutes)

Purpose: This functions as a needs assessment that is particular to the group and can be a guide for future session content.

Session Three

Materials

- Writing paper
- Pens/pencils

Activities

I. Recap of last week (15 minutes)

II. Ice-breaker (10 minutes)

III. **Discussion on the consequences of poor adherence (45 minutes)**

Facilitate a discussion on the possible different outcomes of poor adherence as well as the benefits of good adherence. Take note of members' awareness of:

- Incomplete viral suppression
- Continued destruction of one's immune system
- Disease progression: opportunistic infections
- Emergence of resistant viral strains
- Limited treatment options in our setting
- What level of adherence is needed for optimal viral suppression

IV. **Readiness Ruler/Individual Interviews** (40 minutes)

Hold individual interviews explaining each one's viral load result and discuss with the patient what they envision for their health. Use the readiness ruler in this interview

Purpose: This assesses motivation, confidence and readiness to adhere to medication.

Session Four

Materials

- Writing paper
- Pens/ pencils
- Large chart/ cloth

Activities

I. Recap of last week (15 minutes)

Members are asked to share what they remember from last week and if they have tried anything different regarding their adherence

Also gives opportunity for absent members to catch up on previous weeks

II. Ice-Breaker Chart Game

Have all members stand on a large chart/cloth for 30 seconds. After 30 seconds ask members to get off the chart, fold it and again all stand on the chart for 30 seconds without any members falling off. Keep repeating this till members cannot all stand on the folded chart.

Variation: members keep folding the chart while they remain standing on it

It is usually helpful to ask participants what they have learnt from the ice breaker

III. Narrative exercise

Ask members to take time and reflect and write a narrative on their *Journey with HIV*. Their narrative may include anything they feel is significant to them from the time they knew their status to date. If members ask for help on what they should include always encourage them to write as much as they can on what they feel is relevant. However, these are cues they can build on:

- The experience of getting tested
- Disclosure
- Any sickness/hospitalisations
- Getting started on ART
- Side effects
- When they do not adhere well (barriers to adherence)
- When their adherence is good

*Purpose***:** this gives insight on how members have been processing their HIV status. The emotional, psychological and social issues attached. It also helps give indicators and an understanding of both the barriers and enablers of adherence. Take not of the individual struggles and strength as well as those shared in the group. Begin a discussion on solutions to the barriers of adherence and the strengthening the enablers.

It is very important to be aware that this exercise involves sharing sensitive, personal and private information that at times may be distressing and may cause some anxiety. Reassure members that therapist is there to support them through the process and if individual counselling is needed, that space is available.

Session five

Materials

- Writing paper
- Pens/pencils

Activities

I. Recap of last week (15minutes)

II. Ice-breaker-Pass it on (10 minutes)

Choose an object that is not too big but can fit on someone's neck. The objective of the game is that participants pass the object round without using their hands! If the object is dropped, it must be returned to the previous player, and the game restarts.

III. Question & Answer (35 minutes)

Write down questions that members would have asked in previous sessions. Divide members into teams and ask them to randomly pic a pile of paper with the different questions/barriers to adherence. Each team should discuss and write down at least 3 possible solutions to questions.

IV. Solution Discussion (30 minutes)

Read questions and solutions given by the groups

Facilitate a discussion on solutions given. What does the other team think of solutions? Possible discussion question: are the solutions that have been provided applicable to the group? Is it easier to think of solutions for 'others' but not able to internalize it for themselves.

Homework: try solutions at home the solutions given till next group session.

Session six

Activities

I. Icebreaker (5mins)
Allow members to select an ice breaker of their choice. If different ice breakers are suggested, ask group members to vote.

II. Counsellor's story (30 minutes)/You tube video
A counsellor is asked beforehand to share their journey with HIV. It is hoped that some of their experiences would resonate with group members as well as give some hope to positively living with HIV. Key issues you can ask counsellor to highlight are their feelings and thoughts at being diagnosed with HI, their own struggles with adhering to HAART. How they managed to cope and come to a point of managing adherence.
Such testimonies may help to facilitate thoughts that one's struggles are not individualistic and not manageable. The testimony is also hoped to show positive outcome of adherence to HAART as well a s a healthy self- image.

III. Discussion/Individual sessions (10 minutes)
Allow group members to discuss the testimony/story they have heard as well as ask the counsellors questions.

IV. Discussion and clarification of anything that remains unclear or needs reinforcement. The discussion will also include information on what will occur post Group therapy, in terms of monitoring and notification of post-therapy viral load. Discussion on what it was like thinking of the future; next week, month, year
4

Group therapy experience feedback from the group
Post Viral load testing
Handing back of letters-Dear Future Self .

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