Cross-sectional assessment of virological failure, drug resistance and third-line regimen requirements among patients receiving second-line ART in 3 large HIV-programmes in Kenya, Malawi and Mozambique.

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Introduction

- With ongoing roll-out of routine viral load monitoring, the number of HIV+ patients receiving second-line anti-retroviral therapy (ART) is rapidly increasing in resource-limited settings.
- WHO and national programs need to develop guidelines on third-line therapy.
- More information on second-line drug resistance is needed to guide third-line strategy development: resistance genotyping & individually tailored 3rd line provision versus universally standardised 3rd line regimen.
- Very limited access to new generation drugs with minimal cross-resistance to already used first- and second- line ARVs, - such as darunavir (DRV), the NNRTI etravirine (ETR) and the integrase inhibitor raltegravir (RAL),

Methods

Study design

1. Cross-sectional assessment
- Clinical examination
- Plasma Viral load (VL)
  ⇒ If VL ≥ 500 copies/mL
- Resistance genotyping
- Programmatic enhanced adherence counseling (EAC)
- ART regimen adaptation based on resistance profile

2. Follow up at 6 and 12 months after failure & EAC

Eligibility criteria
- Age ≥ 5 years
- Retained in care
- Standard second-line regimen for ≥ 6 months
- History of failing standard first-line ART regimen

Study sites

Kenya: HIV-outpatient clinic in Homa Bay District Hospital

Malawi: Chiradzulu District, 3 peripheral health centers & District Hospital HIV-clinic

Mozambique: Maputo City, Chamanculo Health District, Alto Mae Referral Health Centre

Sample size: 40-50% of eligible patients per site

Sampling: random (Kenya, Mozambique), convenience (Malawi)

Primary objectives
- To determine the proportion of patients with virological failure
- To describe the drug resistance patterns

Secondary objective
- To assess outcomes at 6 and 12 months after virological failure

Inclusion period: November 2014 - August 2015

HIV viral load in plasma: Kenya and Mozambique: automated real-time PCR using the Cobas Amplicprep/Cobas Taqman HIV-1 test v.2.0 (Roche Diagnostic); Malawi: G2 Generic real-time PCR assay (Biocentric).

HIV-1 resistance genotyping: Subtype determination and resistance was done for patients with VL ≥ 500 cps/ml by sequencing protease and part of reverse transcriptase regions with a WHO-accredited broadly sensitive in-house assay optimised for HIV-1 non B strains. HIV drug resistance scoring was determined by applying the Stanford HIVdb Genotypic Resistance Interpretation Algorithm (Version 7.0, last updated 02/27/14).

Definitions:
- Virological failure: VL ≥ 500 copies/mL
- Second-line ART: boosted protease-inhibitor (PI) combined with two NRTIs.
- Optimized second line regimen: replacement of one or both NRTI drugs due to resistance
- Third-line regimen: replacement of the PI due to PI-resistance, with- or without replacement of NRTI(s)

Results

Participant characteristics at inclusion

	Kenya	Malawi	Mozambique
Eligible, N	705	545	507
Included, N	**355**	**242**	**205**
M/F ratio	0.9	0.8	0.8
Age, years, median [IQR]	38 [30-48]	41 [33-50]	42 [36-47]
Second-line ART, percent			
3TC-TDF-**LPV/r**	72%	-	91%
3TC-ABC-**LPV/r**	17%	9%	6%
3TC-TDF-**ATV/r**	-	82%	-
Years on first-line, median [IQR]	4.7 [3.3-6.4]	3.8 [2.0-5.9]	5.3 [3.3-7.1]
Years on second-line, median [IQR]	2.2 [1.9-3.0]	3.2 [1.1-5.9]	2.3 [1.3-2.6]
CD4 count, cells/µL, median [IQR]	398 [275- 566]	397 [224- 573]	319 [206-432]
WHO clinical stage 1 or 2, percent	98%	94%	97%

- The majority were adults with a median age of 38-42 years.
- In Kenya and Mozambique all patients received a boosted lopinavir (LPV/r)-regimen.
- In Malawi 82% of patients received a boosted atazanavir (ATV/r) regimen.
- Median time on second-line ART at inclusion ranged between 2.2-3.2 years.
- Most participants were in overall good clinical condition at inclusion (WHO stage 1 or 2).

Viral load at inclusion

Participants, N (%) HIV RNA, cps/mL	Kenya 355	Malawi 242	Mozambique 205
<20	252 (71.0)	174 (71.9)	92 (44.9)
>20<500	38 (10.7)	27 (11.2)	52 (25.4)
≥ 500<1000	5 (1.4)	9 (3.7)	6 (2.9)
≥ 1000<10 000	14 (3.9)	12 (5.0)	19 (9.3)
≥ 10 000<100 000	27 (7.6)	10 (4.1)	16 (7.8)
≥ 100 000	19 (5.3)	10 (4.1)	20 (9.7)
≥ 500	65 (18.3)	41 (16.9)	61 (29.7)
≥ 1000	60 (16.9)	32 (13.2)	55 (26.8)
≥ 1000 (≤ 19yrs)	18/60 (30.0)	8/30 (26.6)	10/16 (62.5)

- 13-27% had >1000 cps/mL (WHO-recommended virological failure threshold).
- This translates into overall good suppression (<1000 cps/mL): 83% (Kenya), 87% (Malawi), 73% (Mozambique).
- Virological failure was about twice as high among <19 year patients in all sites
- Resistance genotyping was performed if VL ≥500 cps/mL (N= 65, N=41, N=61)

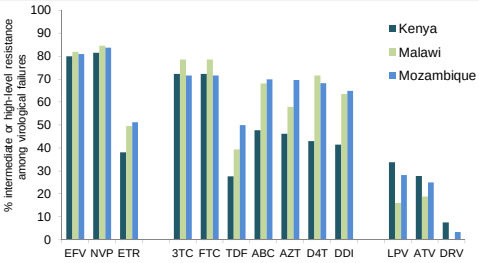
HIV-1 subypes

% of sequenced	Kenya	Malawi	Mozambique
A	25	-	3
A1	51	-	3
C	4	100	90
CRF16_A2D	4	-	-
D	12	-	2
G	-	-	2
URFs *	23	-	-

* Unknown Recombinants constituted by recombination of various subtypes (details not tabulated)

- Malawi: all sequenced strains were subtype C
- Mozambique: 90% were subtype C
- Kenya: few were subtype C, most were A, A1, URFs

Intermediate & high-level resistances by ARV drug



% with resistance *	Kenya Interm.	Kenya high	Malawi Interm.	Malawi high	Mozambique Interm.	Mozambique high
EFV	20	60	23.7	57.9	23.3	58.3
NVP	1.5	80	2.6	81.6	1.7	81.7
ETR	29.2	9.2	31.6	18.4	38.3	13.3
3TC	0	72.3	2.6	76.3	1.7	70
FTC	0	72.3	2.6	76.3	1.7	70
TDF	10.8	16.9	18.4	21	30	20
ABC	18.5	29.2	34.2	34.2	28.3	41.7
AZT	16.7	29.2	21.1	36.8	11.7	58.3
D4T	15.4	27.9	31.6	39.5	13.3	55
DDI	10.8	30.8	31.6	31.6	30	35
LPV	9.2	24.6	8.1	8.1	8.3	20
ATV	10.8	16.9	5.4	13.5	5	20
DRV	7.7	0	0	0	1.7	1.7

Considering prescribed second-line regimen and third-line ARV-options:
- 70-76% had high resistance to 3TC across study sites
- 28-50% had intermediate or high resistance to TDF
- 48-70% had intermediate or high resistance to ABC
- 16-34% had intermediate or high resistance to LPV, and 19-28% to ATV
- Few patients had cross-resistance to DRV: 7.7% (intermediate, Kenya), 3.4% (intermediate or high, Mozambique)
- Considerable cross-resistance to ETR in all sites (38-51%, intermediate/high)

* intermediate and high-level resistance according to Stanford HIVdb
- Kenya: N=65/65 full RT and PR sequences available
- Malawi: N=38/41 RT sequences, N=38/41 PR sequences available
- Mozambique: N=60/61 RT sequences, N=61/61 PR sequences available

ART regimen composition for patients with failure (Kenya)

Simplified second-line, N (%)	24 (37)
3TC-TDF(ABC)-ATV/r	24
Third-line, N (%)	21 (32)
RAL-(ETR)-DRV/r	10
ABC-TDF-**DRV/r**	3
RAL-TDF-**DRV/r**	2
ETR-AZT-**DRV/r**	1
ABC-TDF-ATV/r	2
RAL-ATV/r	2
ETR-TDF-ATV/r	1
Optimized second-line, N (%)	18 (28)
ABC- TDF-ATV/r	13
RAL-AZT (or TDF)-ATV/r	5

- Optimized second-line and third-line regimen were composed of new generation ARV molecules (green) and/or existing PIs and NRTIs (blue), based on resistance profile
- 5 patients required raltegravir for optimized second-line due to broad-spectrum NRTI-resistance

ART regimen requirements among patients with failure

Among VL ≥500 cps/mL patients N (%)	Kenya 65	Malawi 41	Mozambique 61
No change in regimen	-	14 (34) **	25 (41)
Simplified second-line *	24 (37)	3 (7)	-
Optimized second-line	18 (28)	13 (32)	18 (29.5)
Third-line	21 (32)	8 (20)	18 (29.5)
DRV-based regimen *** in absence of PI-resistance	-	3 (7)	-
Change to first-line type regimen	2 (3)	-	-

* change to 1 dose per day- regimen (by replacing LPV/r with ATV/r)
including N=2 with failed resistance genotyping ; * exceptional recommendation in Malawi

- 20-32% of patients with virological failures needed switch to a third-line regimen (major PI resistance)
- 28-32% patients required optimization of their second-line regimen (major NRTi resistance)
- 34-41% were still on an effective regimen
- In Kenya patients with virological failure without major resistance (37%) were changed to a simplified ATV/r-based regimen to support adherence

Six month follow up VL results after failure (Kenya site)

Treatment group N (%)	6 Month FU-VL available	<1000 cps/mL	Deaths	Transfer out
Simplified second-line	17 (71)	10 (58.8)	1 (4.1)	1 (4.1)
Optimized second-line	16 (89)	13 (81.3)	1 (5.5)	-
Third line	15 (71)	12 (80.0)	1 (4.7)	-
Change to first line	2 (100)	0 (0)	-	-

- Overall good VL-suppression (80-81%) six months after treatment switch among patients with optimized second-line or third-line regimen
- Only 58% had VL-suppression among patients receiving simplified second-line
- 3 patients with virological failures died 1-2 months after inclusion (1 of TB diagnosis; 2 at home of unknown cause)

Summary and conclusion

- Overall good virological suppression was achieved on second-line ART, especially in Kenya and Malawi.
- Two-thirds of patients with virological failure required regimen optimization or switch to third-line.
- One third of patients had no major drug resistance , indicating very poor adherence.
- ART Regimen adaptation for patients with failure was through re-cycling of standard available ARVs as well as by use of new generation ARVs.
- Notably higher failure rates found in children and adolescents, highlighting the need for enhanced monitoring.
- Considerable cross-resistance to etravirine was found.
- Preliminary data indicate good short-term outcomes of patients receiving optimized second-line or third-line.

- **Resistance data were essential to inform ART regimen choice of patients with second-line failure**
- **Better access to resistance genotyping and new generation ARVs are urgently needed**

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