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Outcomes After Virologic Failure of First-line ART in South Africa

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BACKGROUND

As the number of patients receiving first-line antiretroviral therapy (ART) has expanded in South Africa, so too have the number experiencing first-line ART regimen failure 1–4. Previously, we reported that specific resistance mutations encountered in South Africa at first ART failure are M184V/I (64%), K103N (51%), thymidine analogue resistance mutations (TAMs; 32%), V106M (19%) and protease inhibitor (PI) resistance mutations (4%) 5. However, there are limited data describing the treatment response after first-line ART failure in resource-limited settings. We report the clinical and virologic outcomes of patients who experienced initial ART regimen failure in KwaZulu Natal, South Africa, after 24-weeks of second-line ART.

METHODS

The Sinikithemba Clinic at McCord Hospital and the iThemba Clinic at St. Mary's Hospital in South Africa provide vertical HIV care for patients from impoverished peri-urban townships. Monitoring follows South African Department of Health recommendations including HIV-1 viral load (assay with detection limit of <50 copies/ml) and CD4 count monitoring six monthly. Clinic counselors provide adherence training before ART initiation and after an elevated viral load.

Study participants

Patients (n=115) were prospectively enrolled adults with a single episode of virologic failure (HIV-1 RNA viral load (VL) ≥1000 copies/mL) during initial combination ART who underwent genotypic resistance testing. Patients with a prior history of dual or monotherapy were not excluded. A subset of patients (n=26; 18% of overall cohort) had resistance testing performed prior to the inception of the prospective cohort in 2005 and were added to the overall cohort. The second-line agents available during the study were: lopinavir/ritonavir (LPV/r; available as gel formulation [Kaletra™]), lamivudine, didanosine (enteric-coated formulation), zidovudine, stavudine, nevirapine and efavirenz. The option to continue an NNRTI-based regimen after initial ART failure was available.

Data collection

Data collected at regimen failure included treatment history, CD4 count, HIV-1 RNA level, WHO stage, hemoglobin and weight. Data collected after 24 weeks of subsequent ART included plasma HIV-1 RNA, CD4 cell count and clinical outcome.

Genotypic resistance testing

Genotypic testing of virus samples was performed at the Nelson Mandela School of Medicine (Durban), using the TRUGENE HIV-1 Genotyping Test (Siemens). Major resistance mutations were previously defined in the initial report describing the cohort.[5]

Statistical analysis

Analyses were performed using SAS software, version 9.1.3 (SAS Institute, North Carolina, USA). All tests of significance were 2-sided; associations with $P < 0.05$ were considered significant. Continuous variables were compared with Wilcoxon rank-sum test; categorical variables with the χ^2 test or Fisher's exact test. An intent-to-treat (ITT) (missing=failure) analysis was performed for the primary outcome of virologic suppression (<50 copies/μl) 24 weeks from enrollment. All outcomes among patients with and without major drug resistance mutations were compared using the χ^2 test and Fisher's exact test. A multivariate logistic regression was performed to determine risk factors associated with mortality after regimen failure.

The study was approved by the ethics committees at McCord and St. Mary's Hospital and by the IRB at Partners HealthCare and Harvard Medical School in Boston, Massachusetts.

RESULTS

Patient characteristics

Between August 2004 and August 2006, 141 patients experienced initial ART virologic failure and underwent genotypic testing. Table 1 shows patient characteristics at regimen failure. At least one major resistance mutation at regimen failure was found for 122 (87%) patients and in 19 (13%) patients had no major resistance mutation detected ("wild-type" genotype).

Virologic and immunological outcomes at 24 weeks

ITT analysis showed that 24 weeks after virologic failure, 99 (70%) patients achieved viral suppression to <400 copies/mL, and 91 (65%) patients to <50 copies/mL. Overall, 50% of patients achieved a 30% improvement in CD4 cell count at 24 weeks follow-up; the median increase in CD4 cell count was 88 cells/μl (IQR 7–168). After 24 weeks, the median 24-week CD4 count was 249 cells/μl (166–343) and only 33% of patients remained with a CD4 cell count of <200 cells/μl.

Mortality and loss-to-follow-up at 24 weeks

The overall mortality among patients 24 weeks after initial ART virologic failure was 6% [95% CI 2-9%], and loss-to-follow-up was 9% [95% CI 4–13%]. Causes of death were tuberculosis (3 patients), gastroenteritis (2), lactic acidosis (1), suspected central nervous system mass (1), and unknown (1). Using a univariate analysis, we compared the characteristics of patients who did not survive 24 weeks of follow-up with those who survived (Table 2). There was a significant (inverse) relationship between the CD4 cell count at regimen failure and 24-week mortality, such that patients with CD4 counts at failure <100 cells/μl experienced higher 24-week mortality compared to patients with CD4 count ≥100 cells/μl (P=0.005). The median CD4 cell count at initial regimen failure among those who died was 70 cells/μl (IQR 27–123) compared to a CD4 cell count of 182 cells/μl (114–260) among patients who survived (P=0.01). Patients who received a boosted PI-containing second-line ART after initial regimen failure experienced a lower mortality over 24 weeks (2%) compared to patients who received NNRTI-based ART (15%) (P=0.004). However, both CD4 count at failure and subsequent regimen type were of borderline significance in multivariate analysis.

Drug resistance at first ART failure

Patients in whom one or more HIV-1 drug resistance mutations were found at virologic failure were compared to patients without resistance mutations detected ("wildtype" genotype). The two groups did not differ by age, gender, ART regimen at failure, or by history of prior dual- or mono-therapy. Patients with drug resistance had a longer median duration of prior initial

ART compared to patients without resistance (13 months versus 8 months) (P<0.05). The median CD4 cell count at initial ART failure was 176 cells/μl (IQR 112–259) in patients with drug resistance and 128 cells/μl [103–222] in patients without resistance (P=0.34). The median HIV-1 RNA viral load at failure among patients with drug resistance was 17,000 copies/mL (IQR 5,500 – 68,264) and 26,766 copies/mL (2,500 – 250,000) in patients without resistance (P=0.4). There was no significant association between the level of viral load at regimen failure and the presence or absence of drug resistance. Patients with drug resistance at regimen failure were more likely to be started on a ritonavir-boosted PI-containing second-line regimen (90%) compared to patients experiencing virologic failure without drug resistance (21%) (P=0.001) as clinicians attempted to optimize regimens.

Viral suppression rates at 24 weeks differed among patients with and without evidence of drug resistance at initial virologic failure. At 24 weeks, 84 of 122 patients (69%) with at least one major mutation achieved viral suppression compared to 7 of 19 patients (37%) without resistant virus (ITT analysis; P=0.01). The median 24-week improvement in CD4 cell count was 89 cells/μl (IQR 12–168) in patients with baseline drug resistance and 34 cells/μl (0–160) in patients without resistant virus (P=0.67). After 24 weeks, 4% of patients with drug resistance and 16% of patients without drug resistance had died (P=0.02).

Effect of drug resistance on boosted PI-based second-line ART outcomes

A total of 107 patients received lopinavir/ritonavir-containing second-line ART. Among patients who initiated a lopinavir/ritonavir-containing regimen, viral suppression at week 24 was achieved in 31 of 39 patients (79%) with at least one TAM as compared to 69 of 102 patients (68%) with no baseline TAMs (P=0.20) and in 4 of 5 patients who initiated lopinavir/ritonavir with ≥3 TAMS. Viral suppression on a lopinavir/ritonavir-containing regimen was achieved in 4 of 5 patients with a K65R mutation. Viral suppression was also achieved in 4 of 5 patients with evidence of one or more major protease mutations. For a fifth patient with at least one major PI resistance mutations, a week 24 plasma HIV-1 RNA measurement was unavailable.

DISCUSSION

This is the first prospective study of second-line ART outcomes in a resource-limited setting. In ITT analysis, after 24 weeks of subsequent ART, 65% of patients achieved viral suppression to <50 copies/mL with a median CD4 cell count improvement of nearly 90 cells/μl. The experience of patients in our cohort compared favorably to that of ART-naïve patients in clinical trials of lopinavir/ritonavir-containing regimens conducted in high-income settings [6].

The use of genotypic drug resistance testing at first ART failure provided important insights. The subgroup of patients in whom no major resistance mutations ("wild-type" genotype) were detected at initial regimen failure experienced higher mortality and greater subsequent loss-to follow-up compared to patients with evidence of drug resistance. A possible explanation for this paradoxical observation is the role of poor adherence, which may not have been resolved before the salvage regimen was initiated. Risk factors for suboptimal adherence have been identified in resource-poor contexts including clinic fees, stigma, regimen complexity, and drug supply interruptions [3, 7–9].

We examined the impact of specific resistance mutations seen at initial virologic failure on salvage outcomes. In this cohort, NRTI resistance mutations (including K65R and TAMs) had minimal impact on 24-week outcomes on boosted PI-based ART. The high pharmacological and genetic barriers to resistance to ritonavir-boosted lopinavir may have allowed patients to overcome the deleterious effects of significant NRTI resistance mutations. However these results should be interpreted with caution given the relatively short follow-up.

The study has several limitations. Second-line ART after virologic failure was informed by genotypic resistance testing and the nucleoside backbone was optimized based upon resistance mutations at initial ART failure. Because this study was not conducted as a controlled clinical trial of the impact of drug resistance testing, we cannot estimate the direct contribution of genotype testing on the outcomes. Second, to maximize the benefit to patients of genotype testing, with limited exceptions (n=4), lopinavir/ritonavir was not given to patients with "wild-type" genotypes, a pattern known to be associated with suboptimal adherence. If suboptimal adherence to initial ART is linked to adherence to subsequent regimens, we may have overestimated the efficacy of lopinavir/ritonavir-containing second-line ART.

In summary, virologic monitoring linked to resistance testing helped demonstrate the efficacy of lopinavir/ritonavir-containing regimens as second-line ART in South Africa. Resistance testing identified a high-risk group without drug resistance who might benefit from increased medication access and/or adherence support. Although regimens that include LPV/r remain more expensive than first-line regimens, even at local access prices, our results suggest that switching to second-line regimens in patients with virologic failure and resistance has substantial and rapid immunological and clinical benefits. Models predict that the prevalence of acquired HIV drug resistance in sub-Saharan Africa will grow substantially over the next decade [10]. Early detection of regimen failure and reductions in the price of boosted PI-based regimens must be prioritized if this patient population is to be effectively treated.

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Table 1

Baseline characteristics of patients with virologic failure during first-line ART with and without evidence of genotypic drug resistance

Characteristic	≥1 Major Resistance Mutation	No Major Mutation Detected
	N=122	N=19
Median age (years) [IQR]	36 (30–42)	43 (35–47) *
Women (%)	51	47
WHO classification (%)		
Class 1	20	21
Class 2	23	21
Class 3	37	42
Class 4	20	16
ART regimen at virologic failure (%)		
D4T – 3TC – EFV	40	63
D4T – 3TC – NVP	7	0
ZDV – 3TC – EFV	29	16
ZDV – 3TC – NVP	12	10
D4T – DDI – EFV	2	0
Other	10	11
Prior dual- or mono-therapy (%)	20	21
Median months of NNRTI-based ART [IQR]	13 (7–20)	8 (6–12) *
Median CD4 count at virologic failure (cells/ul) [IQR] [1]	176 (112–259)	128 (103–221)
CD4 cell count category (%) [1]		
0–49 cells/ul	9	6
50–99 cells/ul	12	17
100–199 cells/ul	36	44
200–349 cells/ul	34	27
≥350 cells/ul	9	6
Median plasma viral load at virologic failure (copies/ml) [IQR] [2]	17,000 (5500–68,264)	26,766 (2500–250,000)
Viral load category (copies/ml) (%) [2]		
400–4,999	22	32
5,000–29,999	38	20
30,000–99,999	23	11
≥ 100,000	17	37
Median hemoglobin (g/dl) [IQR] [3]	13 (11–14)	12 (11–13)
Resistance mutations (%)		
TAM1	14	NA
TAM2	30	NA
K65R	6	NA
Dual Class Resistance (≥1 major NRTI and NNRTI mutation)	21	NA
ART regimen following virologic failure (%)		

Characteristic	≥1 Major Resistance Mutation	No Major Mutation Detected
Lopinavir/ritonavir-based	90	21 **
Non-protease-inhibitor-based	7	63
No subsequent regimen	3	16

Wilcoxon, Chi-square, and Fisher's tests used for two group,

* $p<0.05$

** $p<0.001$

[1] Two patients were missing baseline CD4 cell count

[2] One patient was missing baseline viral load

[3] Eight patients were missing baseline hemoglobin

Table 2

Factors Associated with 24-Week Mortality After Initial ART Virologic Failure

Characteristics	N	- Univariate - 24-Week Mortality no. (%)	P*	- Multivariate - Odds Ratio 95% CI
All patients	141	8 (6)		
Gender				
Female	71	4 (6)		
Male	70	4 (6)	0.98	1.5 (0.2 – 12.3)
History of suboptimal ART				
None	113	7 (6)		
Prior dual or monotherapy	28	1 (4)	0.6	0.4 (0.03 – 7.2)
HIV-1 drug resistance at initial ART failure				
≥1 resistance mutation	122	5 (4)		
No resistance	19	3 (16)	0.06	2.1 (0.1 – 36.2)
Subsequent regimen type[1]				
LPV/r-based ART	114	2 (2)		
NNRTI-based ART	20	3 (15)	0.02	6.3 (0.5 – 83.9)
CD4 cell count at initial ART failure (cells/ul)[2]				
≥100	110	2 (2)		
<100	29	5 (17)	0.005	7.9 (0.8 – 79.7)
HIV-1 RNA viral load at initial ART failure (copies/mL)[3]				
≥ 100,000	27	2 (7)		
< 100,000	113	6 (5)	0.7	4.1 (0.3 – 63.7)
WHO clinical stage at initial ART failure[4]				
Stage III or Stage IV	64	6 (9)		
Stage I or Stage II	47	2 (4)	0.5	0.4 (0.04 – 5.8)

*P-values are for univariate logistic regression model; odds ratio refer to the multivariate model logistic regression model.

[1]Seven patients did not initiate a regimen after virologic failure and three patients from this group died.

[2]One patient who died did not have a CD4 count at first ART failure

[3]One patient who survived did not have a viral load within 8 weeks of first ART failure

[4]Thirty patients who survived did not have a recorded WHO staged at first ART failure