Outcomes after virologic failure of first-line ART in South Africa

Richard A. Murphy[a], Henry Sunpath[b], Zhigang Lu[c], Neville Chelin[b], Elena Losina[c], Michelle Gordon[d], Douglas Ross[e], Aba D. Ewusi[f], Lynn T. Matthews[g], Daniel R. Kuritzkes[h], Vincent C. Marconi[i,j], for the South Africa Resistance Cohort Study Team

Objective: To determine initial 24-week outcomes among prospectively enrolled patients with failure of initial antiretroviral therapy (ART).

Methods: Baseline virologic failure was defined as HIV-1 viral load greater than 1000 copies/ml. Second-line ART was informed by results of genotype testing and selected from agents in the South-African public sector. Twenty-four week endpoints included virologic suppression and mortality.

Results: The cohort consisted of 141 patients (median CD4 cell count and viral load at failure of 173 cells/μl and 17 500 copies/ml). The median prior duration of initial ART was 12.0 months. At least one major resistance mutation was found in 87% of patients.

After 24 weeks of follow-up, intent-to-treat virologic suppression (<50 copies/ml) was 65%, as-treated virologic suppression was 78%, the median CD4 cell count improvement was 88 cells/μl and the mortality was 6%. The median CD4 cell count at initial virologic failure among those who died was 70 cells/μl, compared to 182 cells/μl among patients who survived ($P = 0.01$). Patients with wild-type virus at initial failure ($N = 19$) had inferior outcomes after switch. The presence of nucleoside analogue resistance mutations at failure did not affect early efficacy of boosted-protease inhibitor regimens.

Conclusions: Virologic monitoring linked to resistance testing helped demonstrate the efficacy of lopinavir/ritonavir-containing second-line regimens in South Africa. A switch to second-line regimens in patients with virologic failure and drug resistance has substantial and rapid immunological and clinical benefits. Resistance testing identified a high-risk group without resistance who might benefit from increased medication access and/or adherence support.

AIDS 2010, **24**:1007–1012

Keywords: HIV-1 drug resistance, resource-limited settings, second-line antiretroviral therapy, South Africa, virologic failure

[a]Operational Support Unit, Doctors Without Borders, New York, New York, USA, [b]McCord Hospital, Durban, South Africa, [c]Massachusetts General Hospital, Boston, Massachusetts, USA, [d]Nelson Mandela School of Medicine, Durban, [e]St. Mary's Hospital, Mariannhill, South Africa, [f]Harvard Medical School, [g]Division of Infectious Diseases, Beth Israel Deaconess Medical Center, [h]Section of Retroviral Therapeutics, Brigham and Women's Hospital, Boston, Massachusetts, [i]Infectious Disease Service, San Antonio Military Medical Center, Fort Sam Houston, Texas, and [j]Emory University School of Medicine, Atlanta, Georgia, USA.

Correspondence to Richard A. Murphy, MD, Doctors Without Borders/USA 333 7th Avenue, Second Floor, NY 10001, USA.

E-mail: ramurphy@gmail.com

Received: 23 April 2009; revised: 11 September 2009; accepted: 18 September 2009.

DOI:10.1097/QAD.0b013e3283333639

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 include M184V/I (64%), K103N (51%), thymidine analog resistance mutations (TAMs; 32%), V106M (19%) and protease inhibitor 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 KwaZulu-Natal. Monitoring follows South African Department of Health recommendations including HIV-1 viral load (detection limit of <50 copies/ml) and CD4 cell count monitoring 6-monthly. Clinic counselors provide Adherence training is provided before ART initiation and after any elevated viral load.

Study participants

Patients ($n = 115$) were prospectively enrolled adults with a single episode of virologic failure (HIV-1 RNA viral load $\geq$1000 copies/ml) during initial combination ART who underwent genotypic resistance testing. Patients with a prior history of dual or monotherapy were not excluded. A subset of patients ($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 a non-nucleotide reverse transcriptase inhibitor (NNRTI)-based regimen after initial ART failure was available.

Data collection

Data collected at regimen failure included treatment history, CD4 cell 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, Cary, North Carolina, USA). All tests of significance were two-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 19 (13%) patients had no major resistance mutation detected ('wild-type' genotype).

Virologic and immunological outcomes at 24 weeks

Intent-to-treat analysis showed that 24 weeks after virologic failure, 99 (70%) patients achieved viral suppression to less than 400 copies/ml, and 91 (65%) patients to less than 50 copies/ml. Overall, 50% of patients achieved a 30% improvement in CD4 cell count at 24 weeks follow-up; the median 24-week increase in CD4 cell count was 88 cells/μl [interquartile range (IQR) 7–168]. After 24 weeks, the median 24-week CD4 cell count was 249 cells/μl (166–343) and only 33% of patients remained with a CD4 cell count of less than 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% confidence interval (CI) 2–9%], and loss-to-follow-up was 9% (95% CI 4–13%). Causes of death were tuberculosis (three patients), gastroenteritis (two), lactic acidosis (one), suspected central nervous system mass (one), and

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 ($N=122$)	No major mutation detected ($N=19$)
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 monotherapy (%)	20	21
Median months of NNRTI-based ART (IQR)	13 (7–20)	8 (6–12)*
Median CD4 cell count at virologic failure (cells/μl) (IQR)[a]	176 (112–259)	128 (103–221)
CD4 cell count category (cells/μl) (%)[a]		
0–49	9	6
50–99	12	17
100–199	36	44
200–349	34	27
≥350	9	6
Median plasma viral load at virologic failure (copies/ml) (IQR)[b]	17 000 (5500–68 264)	26 766 (2500–250 000)
Viral load category (copies/ml) (%)[b]		
400–4999	22	32
5000–29 999	38	20
30 000–99 999	23	11
≥100 000	17	37
Median hemoglobin (g/dl) (IQR)[c]	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 (%)		
Lopinavir/ritonavir-based	90	21**
Nonprotease-inhibitor-based	7	63
No subsequent regimen	3	16

Wilcoxon, chi-squared, and Fisher's tests used for two groups. ART, antiretroviral therapy; DDI, didanosine; D4T, stavudine; EFV, efavirenz; IQR, interquartile range; NNRTI, non-nucleotide reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; NVP, nevirapine; ZDV, zidovudine; 3TC, lamivudine.
[a]Two patients were missing baseline CD4 cell count.
[b]One patient was missing baseline viral load.
[c]Eight patients were missing baseline hemoglobin.
*$P<0.05$.
**$P<0.001$.

unknown cause (one). Using 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 cell counts at failure of less than 100 cells/μl experienced higher 24-week mortality compared to patients with CD4 cell count of at least 100 cells/μl ($P=0.005$) and this plausible relationship remained of potential significance in the multivariate model. 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 protease inhibitor-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 cell count at failure and subsequent regimen type were of borderline significance in multivariate analysis of mortality predictors.

Drug resistance at first antiretroviral therapy 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

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)		
Sex				
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[a]				
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/μl)[b]				
≥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)[c]				
≥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[d]				
Stage III or stage IV	64	6 (9)		
Stage I or stage II	47	2 (4)	0.5	0.4 (0.04–5.8)

ART, antiretroviral therapy; LPV/r, lopinavir/ritonavir; NNRTI, non-nucleotide reverse transcriptase inhibitor.
[a]Seven patients did not initiate a regimen after virologic failure and three patients from this group died.
[b]One patient who died did not have a CD4 cell count at first ART failure.
[c]One patient who survived did not have a viral load within 8 weeks of first ART failure.
[d]Thirty patients who survived did not have a recorded WHO staged at first ART failure.
*P values are for univariate logistic regression model; odds ratio refer to the multivariate model logistic regression model.

('wild-type' genotype). The two groups did not differ by age, sex, ART regimen at failure, or by history of prior dual or monotherapy. 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 5500–68 264) and 26 766 copies/ml (2500–250 000) 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 protease inhibitor-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 protease inhibitor-based second-line antiretroviral therapy 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 at least 3 TAMS. Early viral suppression on a lopinavir/ritonavir-containing regimen was achieved in four of five patients with a K65R mutation. Early viral suppression was also achieved in four of five patients with evidence of one or more major protease mutations.

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 less than 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-naive 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 subsequent outcomes. NRTI resistance mutations (including K65R and TAMs) had minimal impact on 24-week outcomes using boosted protease inhibitor-based ART. The high pharmacological and genetic barriers to resistance to ritonavir-boosted lopinavir may have allowed patients to overcome the deleterious effects of major 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 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 protease inhibitor-based regimens must be prioritized if this patient population is to be effectively treated.

Acknowledgements

D.R.K. is a consultant to, or has received research funding from Abbott, Boehringer-Ingelheim, Bristol-Myers Squibb, Gilead, GlaxoSmithKline, Merck and Siemens.

We would like to express our admiration for the work of the iThemba HIV/AIDS Clinic at the St. Mary's Hospital and the Sinikithemba Clinic at McCord Hospital. Important contributions were made by K. Nixon, J. Naidoo and S. Pertab.

Financial support: Grant support from Gilead, NIH (P30 AI60354 to Harvard CFAR and K24 RR16482 to D.R.K.), Harvard Program on AIDS, CDC Cooperative Agreement (U62/CCU123541-01), Schwartz Global Health Fellowship and Elizabeth Glaser Pediatric AIDS Foundation (Project HEART).

SARCS Team Group Authors (Contributed to initial study design, and resistance testing): Bruce D. Walker (Massachusetts General Hospital, Boston, MA), Helga Holst, Janet Giddy, Jane Hampton, Roma Maharaj, Anand Reddi, and Nokuthula Khumalo (McCord Hospital, Sinikithemba Clinic, Durban, South Africa); Steve Carpenter, Kofi Koranteng-Apeagyei, Lorraine Simangele Dube and Jennifer Daily (St. Mary's Hospital, iThemba Clinic, Mariannhill, South Africa); and Nagavelli Padayachi and Alan Smith (Inkosi Albert Luthuli Central Hospital, Nelson Mandela School of Medicine, Durban, South Africa).

References

1. Novitsky V, Wester CW, DeGruttola V, Bussmann H, Gaseitsiwe S, Thomas A, *et al.* **The reverse transcriptase 67N 70R 215Y genotype is the predominant TAM pathway associated with virologic failure among HIV type 1C-infected adults treated with ZDV/ddI-containing HAART in southern Africa.** *AIDS Res Hum Retroviruses* 2007; **23**:868–878.
2. Orrell C, Bangsberg DR, Badri M, Wood R. **Adherence is not a barrier to successful antiretroviral therapy in South Africa.** *AIDS* 2003; **17**:1369–1375.
3. Mills EJ, Nachega JB, Buchan I, Orbinski J, Attaran A, Singh S, *et al.* **Adherence to antiretroviral therapy in sub-Saharan Africa and North America: a meta-analysis.** *J Am Med Assoc* 2006; **296**:679–690.
4. Nachega JB, Hislop M, Dowdy DW, Chaisson RE, Regensberg L, Maartens G. **Adherence to nonnucleoside reverse transcriptase inhibitor-based HIV therapy and virologic outcomes.** *Ann Intern Med* 2007; **146**:564–573.

5. Marconi VC, Sunpath H, Lu Z, Gordon M, Koranteng-Apeagyei K, Hampton J, *et al*. **Prevalence of HIV-1 drug resistance after failure of a first highly active antiretroviral therapy regimen in KwaZulu Natal, South Africa.** *Clin Infect Dis* 2008; **46**:1589–1597.
6. Walmsley S, Bernstein B, King M, Arribas J, Beall G, Ruane P, *et al*. **Lopinavir-ritonavir versus nelfinavir for the initial treatment of HIV infection.** *N Engl J Med* 2002; **346**:2039–2046.
7. Nachega JB, Stein DM, Lehman DA, Hlatshwayo D, Mothopeng R, Chaisson RE, Karstaedt AS. **Adherence to antiretroviral therapy in HIV-infected adults in Soweto, South Africa.** *AIDS Res Hum Retroviruses* 2004; **20**:1053–1056.
8. Hardon AP, Akurut D, Comoro C, Ekezie C, Irunde HF, Gerrits T, *et al*. **Hunger, waiting time and transport costs: time to confront challenges to ART adherence in Africa.** *AIDS Care* 2007; **19**:658–665.
9. Laniece I, Ciss M, Desclaux A, Diop K, Mbodj F, Ndiaye B, *et al*. **Adherence to HAART and its principal determinants in a cohort of Senegalese adults.** *AIDS* 2003; **17 (Suppl 3)**:S103–108.
10. Blower S, Bodine E, Kahn J, McFarland W. **The antiretroviral rollout and drug-resistant HIV in Africa: insights from empirical data and theoretical models.** *AIDS* 2005; **19**:1–14.