Lopinavir/ritonavir monotherapy after virologic failure of first-line antiretroviral therapy in resource-limited settings

John A. Bartlett[a,b], Heather J. Ribaudo[c], Carole L. Wallis[d], Evgenia Aga[c], David A. Katzenstein[e], Wendy S. Stevens[f], Michael R. Norton[g], Karin L. Klingman[h], Mina C. Hosseinipour[i], John A. Crump[a,b], Khuanchai Supparatpinyo[j], Sharlaa Badal-Faesen[f], Beatrice A. Kallungal[k] and Nagalingeswaran Kumarasamy[l]

Objective: To evaluate virologic response rates of lopinavir/ritonavir (LPV/r) monotherapy as second-line antiretroviral treatment (ART) among adults in resource-limited settings (RLSs).

Design: An open-label pilot study of LPV/r monotherapy in participants on first-line nonnucleoside reverse transcriptase inhibitor three-drug combination ART with plasma HIV-1 RNA 1000–200 000 copies/ml.

Methods: Participants were recruited from five sites in Africa and Asia within the AIDS Clinical Trials Group (ACTG) network. All participants received LPV/r 400/100 mg twice daily. The primary endpoint was remaining on LPV/r monotherapy without virologic failure at week 24. Participants with virologic failure were offered addition of emtricitabine and tenofovir (FTC/TDF) to LPV/r.

Results: Mutations associated with drug resistance were encountered in nearly all individuals screened for the study. One hundred and twenty-three participants were enrolled, and 122 completed 24 weeks on study. A high proportion remained on LPV/r monotherapy without virologic failure at 24 weeks (87%). Archived samples with HIV-1 RNA levels less than 400 copies/ml at week 24 ($n = 102$) underwent ultrasensitive assay. Of these individuals, 62 had levels less than 40 copies/ml and 30 had levels 40–200 copies/ml. Fifteen individuals experienced virologic failure, among whom 11 had resistance assessed and two had emergent protease inhibitor mutations. Thirteen individuals with virologic failure added FTC/TDF and one individual added FTC/TDF without virologic failure. At study week 48, 11 of 14 adding FTC/TDF had HIV-1 RNA levels less than 400 copies/ml.

Conclusion: In this pilot study conducted in diverse RLS, LPV/r monotherapy as second-line ART demonstrated promising activity.

AIDS 2012, **26**:1345–1353

[a]Duke University Medical Center, Durham, North Carolina, USA, [b]Kilimanjaro Christian Medical Centre, Moshi, Tanzania, [c]Statistical Data Analysis Center, Harvard University, Boston, Massachusetts, USA, [d]Lancet Laboratories, Johannesburg, South Africa, [e]Stanford University Medical Center, Stanford, California, USA, [f]University of Witswatersrand and National Health Laboratory Service, Johannesburg, South Africa, [g]Abbott, Abbott Park, Illinois, USA, [h]Division of AIDS, National Institute for Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, USA, [i]University of North Carolina, Chapel Hill and UNC Project, Lilongwe, Malawi, [j]Research Institute for Health Sciences, Chiang Mai University, Chiang Mai, Thailand, [k]Social and Scientific Systems, Silver Spring, Maryland, USA, and [l]YRGCARE Medical Centre, Voluntary Health Services, Chennai, India.

Correspondence to John A. Bartlett, Box 3238, Duke University Medical Center, Durham, NC 27710, USA.
Tel: +1 919 681 8043; fax: +1 919 681 7748; e-mail: jab5@duke.edu
Received: 23 December 2011; revised: 24 February 2012; accepted: 14 March 2012.

DOI:10.1097/QAD.0b013e328353b066

Keywords: lopinavir/ritonavir monotherapy, resource-limited settings, second-line antiretroviral therapy

Introduction

More than 5 million HIV-1-infected persons are currently receiving antiretroviral therapy (ART) globally (http://www.globalhealthfacts.org/data/topic/map.aspx?ind=10), resulting in dramatic reductions of HIV-1-related morbidity and mortality. The WHO recommends a simplified public health approach to ART prescription to reach HIV-1-infected persons who live in resource-limited settings (RLSs) (http://whqlibdoc.who.int). First-line ART is suggested with a nonnucleoside reverse transcriptase inhibitor (NNRTI) and two nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), representing potent, safe and relatively inexpensive regimens. Virologic suppression rates on first-line ART commonly exceed 80% at 1 year on treatment among patients who are retained in care [1]. In the event of first-line ART failure, second-line ART is available in many RLSs, which consists of a boosted protease inhibitor-containing regimen in combination with two NRTIs. Second-line ART also provides high rates of plasma HIV-1 RNA suppression at 1 year, with approximately 75% of patients achieving levels less than 400 copies/ml if they remain in care [1].

Despite the therapeutic successes of ART, considerable challenges remain. Monitoring of plasma HIV-1 RNA levels is not available in most RLS, and as a result, first-line ART failure is often identified by either immunological or clinical changes that may occur long after loss of virologic suppression. The delay in identifying ART failure has been shown to lead to the accumulation of complex resistance profiles that impact susceptibility to NRTIs and their future use [2–6]. It is unknown to what extent the inclusion of NRTI in second-line ART regimens contributes to antiretroviral activity given these observations. In the worst-case scenario, NRTIs may only expose patients to the increased risk of drug-related toxicities and add cost to second-line ART.

Previous studies of boosted protease inhibitor monotherapy in treatment-naive HIV-1-infected persons have suggested that most adherent patients can achieve significant virologic suppression, albeit less robust than that achieved with three-drug, multiclass drug combinations [7,8]. Interestingly, despite observations of low-level viremia among boosted protease inhibitor monotherapy recipients, studies to date have rarely identified the evolution of drug resistance mutations in the HIV protease gene [9]. In RLSs with limited availability of alternative antiretroviral medications and extensive NRTI resistance following first-line virological failure, boosted protease inhibitor monotherapy could represent a viable option for second-line ART. The A5230 study of the AIDS Clinical Trial Group (ACTG) was designed to evaluate the activity and tolerability of second-line ART with lopinavir/ritonavir (LPV/r) monotherapy in a pilot study among persons experiencing virologic failure on a first-line regimen containing an NNRTI and two NRTIs. The study has a 24-week primary endpoint with continued long-term follow-up to 2 years; here we present the primary analysis results.

Methods

Study design

ACTG A5230 is an open-label, multicenter pilot study of LPV/r monotherapy in individuals failing a first-line regimen containing an NNRTI and two NRTIs, (NCT00357552). The study was conducted at five Clinical Research Sites, including three sites in Africa (Lilongwe, Malawi; Moshi, Tanzania; and Johannesburg, South Africa) and two sites in Asia (Chiang Mai, Thailand; and Chennai, India). The primary objective of the study was to demonstrate that LPV/r monotherapy provides at least a 65% virologic response rate in this patient population, defined as achieving and maintaining HIV-1 RNA levels less than 400 copies/ml for at least 24 weeks while on LPV/r monotherapy.

Eligible individuals

Eligible individuals had documented HIV-1 infection with two methods of antibody and/or antigen or nucleic acid detection, and were 18 years or older. They must have received a first-line regimen containing an NNRTI and two NRTIs for at least 6 months continuously, and have a plasma HIV RNA-1 level between 1000 and 200 000 copies/ml within 30 days of study entry. Eligible individuals were also required to have the following laboratory values: hemoglobin more than 8 mg/dl, platelets more than 50 000/μl, estimated creatinine clearance more than 60 ml/min, aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase less than three times the upper limit of normal, and total bilirubin less than 2.5 times the upper limit of normal. Pregnant or breastfeeding women and persons with prior protease inhibitor use, a serious medical condition within the previous 14 days, active substance abuse or evidence of chronic hepatitis B infection as documented by a positive hepatitis B surface antigen result were excluded. All individuals provided written informed consent.

Study evaluations

For the first 24 weeks following LPV/r treatment initiation, individuals returned at weeks 2 and 4, and then every 4 weeks until week 24. At every study visit, a clinical assessment, list of concomitant medications, adherence interview using the ACTG Adherence Questionnaire [10] and pill count were performed. Serum chemistries, liver enzymes and hematology values were measured at weeks 4, 8, 12 and 24. Fasting lipid panels were done at study entry, and weeks 12 and 24. Plasma HIV-1 RNA levels were measured at study entry, and weeks 12, 16, 20 and 24. $CD4^+$ cell counts were measured at study entry, and weeks 16 and 24. All laboratories participated in international quality assurance testing. With the exception of samples drawn at week 2 and 4, quantitation of HIV-1 RNA levels in A5230 was performed onsite in real time using a HIV-1 RNA assay approved by US Food and Drug Administration; week 2 and 4 samples were tested at the time of the week 12 sample. In a posthoc analysis, all week 24 samples with plasma HIV RNA-1 less than 400 copies/ml underwent further evaluation with the Abbott *m*2000*rt* RealTi*me* assay (Abbott Laboratories, Abbott Park, Illinois USA; lower limit of detection 40 copies/ml) in one of three designated laboratories. Samples for genotypic resistance testing were collected on all individuals at the screening visit. Resistance testing was performed in-batch at two regional laboratories using ViroSeq (Celera Diagnostics, California, USA) as per manufacturer's instructions. Briefly, a 1.7 kb amplicon was generated by a reverse transcriptase-initiated PCR encompassing the entire protease and partial reverse transcriptase. Sequencing was performed with an ABI Prism 3100-*Avant* Genetic Analyzer (Applied Biosystems, Pleasanton, California, USA). HIV-1 subtype and drug resistance were determined from protease transcriptase and reverse transcriptase sequences and analyzed using the Stanford Algorithm Database [11,12].

Study interventions

All individuals received fixed dose combination (FDC) LPV/r 400/100 mg twice daily. If patients developed virologic failure, FDC emtricitabine 200 mg/tenofovir 300 mg (FTC/TDF) daily was added to LPV/r. Concomitant treatment with rifampin and other medications listed in the LPV/r package insert was prohibited.

Primary endpoint

The primary endpoint was remaining on LPV/r monotherapy without virologic failure at week 24. Virologic failure was defined as failure to suppress plasma HIV-1 RNA to less than 400 copies/ml by week 24 or confirmed rebound of plasma HIV-1 RNA levels to more than 400 copies/ml after confirmed suppression to less than 400 copies/ml. With some exceptions per protocol, discontinuation of LPV/r monotherapy was considered as a primary endpoint; exceptions included discontinuation for rifampin-based tuberculosis treatment, addition of antiretroviral drugs due to pregnancy and death with HIV-1 RNA levels less than 400 copies/ml.

Sample size

The target sample size of 120 individuals was chosen to provide at least 90% power to show that LPV/r monotherapy could provide at least a 65% success rate over 24 weeks. This assumed that the true success rate of the strategy in this population is 76%, and used a one-sided type I error rate of 5%.

Statistical analysis

Baseline characteristics of the study population were compared across sites using nonparametric *k*-sample tests. The proportion of patients remaining on LPV/r monotherapy without virologic failure at week 24 was estimated with an exact 95% confidence interval. The proportion of patients with plasma HIV-1 RNA levels less than 400 copies/ml over time was estimated using intent-to-treat methods with missing/off treatment evaluation ignored (M = I) and considered as failure ($\geq$400 copies/ml) (M = F); as treated analyses were also performed. Exact logistic regression was used to assess factors associated with the probability of failure; prior use of efavirenz (EFV) vs. nevirapine (NVP), prior therapy duration (>3 years, 1–3 years and <1 year), sex, baseline $CD4^+$ cell count (<200 vs. >200 cells/μl), baseline plasma HIV-1 RNA levels (<10 000 vs. >10 000 copies/ml) and NRTI resistance (defined as K65R or thymidine analogue mutations vs. no NRTI resistance). In unplanned posthoc analyses, baseline HIV-1 RNA levels and rates of failure are described. All analyses were restricted to data from study entry to the scheduled week 24 visit. In order to provide information on the consequences of LPV/r monotherapy failure, the HIV-1 RNA and $CD4^+$ cell count responses and resistance findings through week 48 are included from patients reaching a primary failure endpoint.

Results

Baseline characteristics

One hundred and twenty-three patients entered the study; their baseline characteristics are described in Table 1. The majority of them were women. The most common first-line ART regimen was NVP-containing, lamivudine (3TC)-containing and stavudine (d4T)-containing regimens ($n = 63$, 51%), and the majority of patients had received ART for more than 3 years ($n = 70$, 57%). The median plasma HIV-1 RNA level was 4.34 $\log_{10}$ copies/ml and 21 patients (17%) had levels more than 100 000 copies/ml. The median $CD4^+$ cell count at study entry was 164 cells/μl and 70 patients (57%) had counts less than 200 cells/μl. Screening plasma HIV-1 RNA levels, the proportion of patients with baseline $CD4^+$ cell counts below 100 cells/μl and the proportion

Table 1. Baseline characteristics.

	Total ($N=123$)
Age (years)	
Median (Q1–Q3)[a]	39 (33–45)
Sex	
Female	70 (57%)
Prior regimen[b]	
TDF + 3TC + NVP	4 (3%)
ZDV + 3TC + EFV	8 (7%)
ZDV + 3TC + NVP	26 (21%)
d4T + 3TC + EFV	21 (17%)
d4T + 3TC + NVP	63 (51%)
ddI + 3TC + NVP	1 (1%)
Total ARV exposure	
Median (Q1–Q3)[a]	3 (2–4)
<1 years	9 (7%)
1 to <3 years	44 (36%)
>3 years	70 (57%)
HIV-1 RNA [$\log_{10}$ (copies/ml)]	
Median (Q1–Q3)[a]	4.34 (3.75–4.92)
<3.00	4 (3%)
3.00–3.99	38 (31%)
4.00 to <4.99	60 (49%)
5.00 to <5.29	14 (11%)
≥5.3	7 (6%)
CD4 cell count (cells/μl)	
Median (Q1–Q3)[a]	164 (82–268)
<50	19 (15%)
50–199	51 (41%)
200–349	39 (32%)
≥350	14 (11%)

3TC, lamivudine; D4T, stavudine; DDI, didanosine; EFV, efavirenz; NVP, nevirapine; TDF, tenofovir; ZDV, zidovudine.
[a]Q1 and Q3 represent the 25th and 75th percentiles, respectively.
[b]Prior regimen: most recent regimen before enrolling in A5230.

of patients meeting WHO $CD4^+$ cell count criteria for regimen failure [13] were found to differ by site ($P=0.016$, $P<0.001$, $P=0.002$, $P<0.001$, respectively), with patients from the South African site having significantly lower values for each parameter.

Baseline resistance and subtyping

From the 114 screening samples that were successfully sequenced, nearly all patients had mutations associated with drug resistance. Most patients were found to have mutations associated with reduced susceptibility to 3TC ($n=108$; 95%), EFV ($n=112$; 98%) and NVP ($n=112$; 98%). Based on genotyping of the protease transcriptase and partial reverse transcriptase, HIV-1 subtype C was found to be the most prevalent (66%), followed by AE (19%), A1 (7%) and D (6%).

Patient disposition

Of 123 patients who entered the study, 122 completed 24 weeks of study follow-up; one patient died from a myocardial infarction deemed unrelated to treatment with a plasma HIV-1 RNA level less than 400 copies/ml at week 20. All patients remained on study treatment (LPV/r monotherapy or LPV/r with FTC/TDF) throughout the 24-week primary endpoint.

Virologic responses

At week 24, 107 (87%) patients remained on LPV/r monotherapy without virologic failure (95% confidence interval 80–92). From week 16, more than 80% of patients had plasma HIV-1 RNA levels less than 400 copies/ml regardless of how missing data and premature treatment discontinuations were analyzed (Fig. 1). Of note, 95 of 102 patients (93%) entering with baseline plasma HIV-1 RNA levels less than 100 000 copies/ml had achieved HIV-1 RNA less than 400 copies/ml by week 16 compared with 19 of 21 (90%) with baseline levels more than 100 000 copies/ml. One hundred and two patients had plasma HIV-1 RNA levels less than 400 copies/ml at week 24. Among these 102 patients (panel within Fig. 1), 62 (61%) had plasma HIV-1 RNA less than 40 copies/ml, 30 (29%) had levels 40–200 copies/ml, seven (7%) had levels 201–400 copies/ml

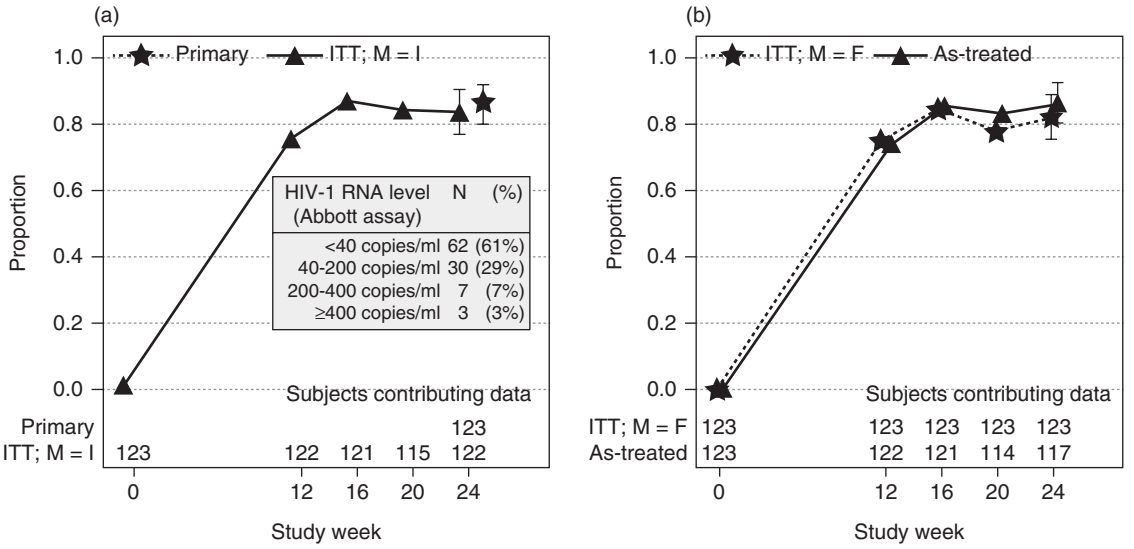

Fig. 1. Proportion of patients with HIV-1 RNA levels less than 400 copies/ml.

and three (3%) had levels more than 400 copies/ml (respectively, 413, 489 and 1712 copies/ml).

Virologic failures

Fifteen patients met the criteria for virologic failure at week 24, and an additional patient had FTC/TDF added without virologic failure (total failures 16, 13%) (Fig. 2). Failure occurred in six of 21 patients (29%) entering with baseline plasma HIV-1 RNA levels more than 100 000 copies/ml; in contrast, of the 102 patients with baseline plasma HIV-1 RNA levels less than 100 000 copies/ml, 10 (10%) met virologic failure criteria. Among the 15 patients with virologic failure, protease transcriptase and reverse transcriptase sequences were obtained from 11 (73%); one individual had insufficient sample available and three samples could not be sequenced [HIV-1 RNA level <1000 copies/ml (two patients) and 1330 copies/ml (one patient)]. Protease transcriptase mutations not observed at study entry were identified in two patients; V82F in one and L33F, M46I, I54V, V82A and L90M in the second (Table 2). With regard to NRTI-associated mutations at the time of virologic failure, eight patients had between one and six mutations that were no longer detectable compared with mutations detected at screening. Five patients gained one NRTI-associated mutation from study entry to virologic failure. From study entry to virologic failure, no NNRTI-associated mutations were gained by any of the patients and in six patients NNRTI mutations were no longer detectable.

Predictors of virologic failure

In unadjusted and adjusted analyses, no associations between the potential predictors and virologic failure were apparent ($P > 0.10$).

Treatment intensification

By study week 48, 14 (88%) of the 16 primary endpoint failures intensified their treatment with FTC/TDF and 11 (79%) of 14 suppressed to less than 400 copies/ml following intensification (Fig. 2). Among the three patients who failed to suppress, one (33%) had newly emergent protease inhibitor-associated mutations at the time of virologic failure. Of the patients who suppressed to less than 400 copies/ml on treatment intensification, all were suppressed at study week 48. Table 2 lists the resistance profiles for patients at screening and at virologic failure, categorized by receipt of treatment intensification and virologic response. Among the 11 patients who suppressed HIV-1 RNA to less than 400 copies/ml, seven had at least one major NRTI-associated resistance mutation that could no longer be detected by population-based sequencing. The two patients who did not suppress HIV-1 RNA levels to less than 400 copies/ml either maintained the same number of NRTI-associated resistance mutations or gained a new mutation.

$CD4^+$ cell count responses

$CD4^+$ cell counts increased from a median (Q1–Q3) of 164 (82–268) cells/μl at study entry to a median of 268 (169–404) cells/μl at week 24. Among patients remaining on LPV/r monotherapy without primary endpoint failure at week 24, the median $CD4^+$ cell count increase over 24 weeks was 102 (46–157) cells/μl compared with 111 (35–172) cells/μl for the primary endpoint failures.

Adherence

Adherence levels were generally high throughout the 24 weeks; by self-report, 79–90% of patients responded that they had never missed a dose of study medication at each adherence assessment. Hypothesis-testing statistical

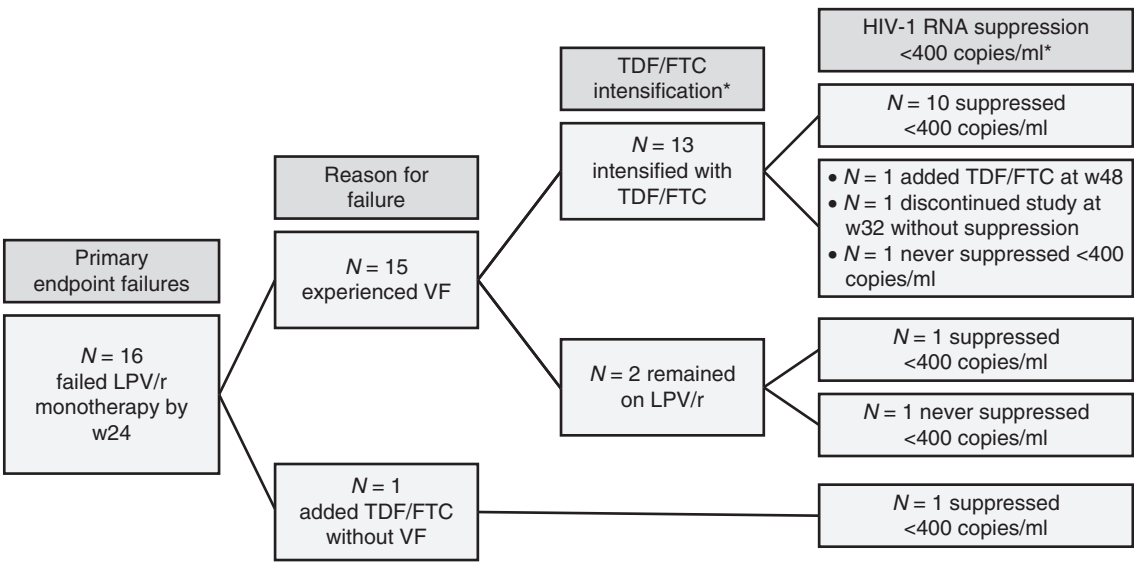

Fig. 2. Primary endpoint failures.

Table 2. Resistance profiles at screening and virologic failure time points, for the patients who failed virologically.

			Resistance-associated mutations and polymorphisms[a]		
ID	Visit	VL[b]	PI	NRTI	NNRTI
Did not intensify LPV/r monotherapy					
1	SCR	30 344	Not detected	K65R, D67N, Q151M, Y181C	K103N, Y181C
	VF	618	Not detected	M184V	K103N, E138Q(n/r)
2	SCR	7 916	'Failed sequencing'		
	VF	637	'Could not be amplified'		
Did not suppress to <400 copies/ml following LPV/r monotherapy intensification					
3	SCR	30 100	L89M(n/r)	Not detected	Not detected
	VF	39 500	L89M(n/r)	G333D	Not detected
4	SCR	60 137	L10F, E35L(n/r), L89M(n/r), L90M	M184V, T215Y	K103N, E138Q(n/r), K238T
	VF	37 020	L10F, L33F, E35F(n/r), M46I, I54V, V82A, L89M(n/r), L90M	M184V, T215Y	K103N, E138Q(n/r), K238T
Suppressed to <400 copies/ml following LPV/r monotherapy intensification					
5	SCR	10 800	E35L(n/r), L89M(n/r)	M184V	K103N, P225H
	VF	972	E35L(n/r), L89M(n/r)	M184V	K103N, P225H
6	SCR	151 566	L89M(n/r)	Not detected	K103N
	VF	7 214	'Insufficient sample'		
7	SCR	126 659	E35L(n/r), T74S, L89M(n/r)	D67N, K70R, Y181C, M184V, T215I, K219E	V108I, V179I(n/r), Y181C
	VF	1 193	E35L(n/r), T74S, L89M(n/r)	D67N, K70R, Y181C, M184V, K219E, G333D	V108I, V179I(n/r), Y181C, Y318ND(n/r)
8	SCR	55 570	L89M(n/r)	M41L, D67N, L74I, V118I, M184I, L210W, T215Y, K219R	K103N
	VF	1 330	'Could not be sequenced'		
9	SCR	186 324	E35L(n/r), L89M(n/r)	Y181C, M184V, G333D	V179I(n/r), Y181C, Y318ND(n/r)
	VF	16 860	E35L(n/r), L89M(n/r)	Y181C, M184V	V179I(n/r), Y181C
10	SCR	194 847	A71T	K65R, Y181C, M184V	K101E, Y181C, G190A
	VF	699	L33V(n/r), A71T	Not detected	Not detected
11	SCR	6 831	E35L(n/r)	M184V	K103N, V179I(n/r), K238T
	VF	736	'Could not be amplified'		
12	SCR	124 025	L10I, E35L(n/r), L89M(n/r)	M41L, D67N, V75M, Y181C, M184V, L210W, T215Y	V179I(n/r), Y181C, G190A
	VF	5 309	L10I, E35L(n/r), V82F, L89M(n/r)	Not detected	V179I(n/r)
13	SCR	5 001	K43R(n/r), A71T	V75A, V118AV(n/r), M184V	K101E, E138A(n/r), G190S
	VF	1 358	K43KR(n/r), A71T	Not detected	E138A(n/r)
14	SCR	40 907	L10I, E35L(n/r), L89M(n/r)	M184V, T215Y	K103N, V179I(n/r)
	VF	6 113	L10ND(n/r), V11ND(n/r), E35L(n/r), L89M(n/r)	G333D	V179I(n/r)
15	SCR	101 655	Not detected	A62V, T69N, K70N, V75T, Y181C, M184V	K101E, Y181C, G190A
	VF	904	Not detected	G333D	Y318ND(n/r)

LPV/r, lopinavir/ritonavir; NNRTI, nonnucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; SCR, at screening; VF, at virologic failure.
[a]Mutation mixtures have been converted to mutations.
[b]HIV-1 RNA (copies/ml) quantified from a sample drawn at the same time as the sequencing sample.

analyses were not performed, but there appeared to be no differences in reported adherence between patients who were virologic successes compared with those who were failures.

Safety and tolerability

Thirty-one patients (25%) experienced grade 3 or 4 adverse events during LPV/r monotherapy (Table 3). The most common events included lipid elevations (one patient with grade 3 fasting triglycerides, six with grade 4 fasting triglycerides, five with grade 3 fasting low-density lipoprotein cholesterol and five with grade 3 fasting total cholesterol), elevations in fasting blood sugar (two patients with grade 3 and four with grade 4) and abnormalities in serum phosphorus (five patients with grade 3 hypophosphatemia).

Discussion

This pilot study, conducted at five geographic sites with multiple HIV-1 subtypes represented, demonstrates promising antiretroviral activity of LPV/r monotherapy as second-line ART in NNRTI-experienced HIV-infected persons. Over 24 weeks, 87% of patients remained on LPV/r monotherapy with plasma HIV-1 RNA levels suppressed to less than 400 copies/ml with the lower limit on the estimated efficacy of the regimen of 80%, exceeding our target threshold of 65%. Among patients who developed virologic failure, treatment intensification with FDC FTC/TDF resulted in suppression to less than 400 copies/ml over 48 weeks for the majority of them.

As the number of HIV-1-infected persons on first-line ART grows, inevitably some will fail and need second-line ART. Currently, the numbers of patients switching to second-line regimens are relatively low, estimated at 3–5% [14–16], although it is likely that 15–25% of HIV-infected persons on first-line ART for at least 12 months have detectable plasma HIV-1 RNA [16]. When plasma HIV-1 RNA monitoring is available, it does lead to earlier switches to second-line ART [16], and better outcomes have been associated with RNA less than 1000 copies/ml at the time of switching [17]. Unfortunately, in many RLSs, RNA measurement is not available and first-line ART failure is recognized late, with the consequence of increasing patient numbers with extensive NRTI and NNRTI resistance mutations.

Other studies of second-line ART in RLS have demonstrated similar rates of virologic suppression with use of LPV/r–NRTI [17–19]. One study examined outcomes in 328 patients receiving LPV/r, zidovudine and didanosine as second-line ART; plasma HIV-1 RNA levels were available for 262 patients and 203 (77%) had levels less than 400 copies/ml after 1 year, comparable to our results with LPV/r monotherapy [17]. Another study of second-line ART in 141 South African patients included the use of LPV/r and genotypic resistance testing to guide the choice of the two NRTIs [18]. Baseline characteristics of the South African study population were similar to that of ACTG A5230 patients with a median plasma HIV-1 RNA of 17 000 copies/ml and 17% with RNA more than 100 000 copies/ml, although NRTI and NNRTI resistance was not as extensive as observed in our population. Seventy-seven percent of these patients suppressed to less than 400 copies/ml and 65% to less than 50 copies/ml at week 24, similar to the 87% of patients with less than

Table 3. Grade 3 or 4 adverse events during lopinavir/ritonavir monotherapy that were reported for at least 2% of the study patients.

	Number of patients by DAIDS toxicity grade		
Sign/symptom/laboratory event	3	4	Total patients [*n* (%)]
Total triglycerides (fasting)	1	6	7 (6)
Fasting blood sugar	2	4	6 (5)
LDL (fasting)	5	0	5 (4)
Phosphorus	5	0	5 (4)
Total cholesterol (fasting)	5	0	5 (4)
SGOT	1	2	3 (2)
Absolute neutrophil count	2	1	3 (2)
Diarrhea/loose stools	2	1	3 (2)
Total bilirubin	2	1	3 (2)
Hemoglobin	3	0	3 (2)
SGPT	0	2	2 (2)
Bicarbonate	1	1	2 (2)
Potassium	1	1	2 (2)
Cachexia/wasting/weight loss	2	0	2 (2)

Table reflects the number of patients reporting each category of symptom/laboratory event: 3, severe; 4, life-threatening; 5, death. For each patient, the worst grade for each event category is reported. DAIDS, Division of AIDS; LDL, low-density lipoprotein; SGOT, serum glutamic oxaloacetic transaminase; SGPT, serum glutamic pyruvic transaminase.

400 copies/ml and 50% (62/123) with less than 40 copies/ml observed in ACTG 5230. A third study of second-line ART with LPV/r, zidovudine, lamivudine and TDF among 109 persons in Malawi found that 85% of those surviving at 1 year had plasma HIV-1 RNA levels less than 400 copies/ml [19].

Previous trials of LPV/r monotherapy have observed findings of low-level viremia in first-line regimens, and randomized trials have demonstrated such viremia is significantly greater with monotherapy compared to three-drug LPV/r-containing regimens [7,8]. When week 24 samples for patients remaining on LPV/r monotherapy with plasma HIV-1 RNA levels less than 400 copies/ml in A5230 were re-tested using an ultrasensitive assay, low-level viremia was detected in 39% with the majority of samples measured in the 40–200 copies/ml range. The significance of low-level viremia is uncertain, with other trials of LPV/r maintenance monotherapy suggesting that it may remain stable for prolonged periods [20,21]. Numerous studies have demonstrated that resistance mutations in the protease gene are infrequent in persons receiving boosted protease inhibitors [22], even as monotherapy, despite this low-level viremia [9,21]. Similar observations were made in ACTG A5230 – only 7% (2/16) patients virologically failing LPV/r monotherapy had protease mutations that were not observed at study entry.

A recent Thai study randomized patients failing first-line ART to either LPV/r monotherapy or LPV/r, FTC and TDF, and was conducted simultaneously with ACTG A5230 [23]. They observed that the proportion of patients with viremia at levels 50–400 cells/ml after 48 weeks was significantly higher in the monotherapy arm (14 vs. 3%), leading them to conclude that three-drug second-line regimens were superior. A second randomized trial of LPV/r maintenance monotherapy vs. combination therapy (SARA trial) was conducted in Uganda and Zimbabwe among 192 patients who had received 24 weeks of second-line combination therapy including LPV/r [24]. Plasma HIV-1 RNA was not monitored in real-time, but testing of archived specimens revealed that the combination therapy group had greater virologic suppression at week 24 (77 vs. 60%, <50 copies/ml). Genotypic resistance testing on 10 monotherapy patients with plasma HIV-1 RNA levels more than 1000 copies/ml revealed that a minority of patients had new protease mutations at the time of failure. Treatment intensification was not included in the SARA trial design, and the availability of real-time plasma HIV-1 RNA monitoring and intensification represent an important distinguishing feature of ACTG A5230.

The short-term virologic responses to FTC/TDF intensification in ACTG A5230 patients are encouraging, and similar responses were observed in the Thai trial in which eight of 17 patients in the LPV/r monotherapy arm suppressed to less than 50 copies/ml following intensification with FTC/TDF [23] and in trials of LPV/r monotherapy when NRTIs were added in patients with low-level viremia [21,22]. The strategy of LPV/r monotherapy followed by treatment intensification in the event of virologic failure may be critically important for future studies of LPV/r monotherapy. However, this strategy does impose the need for plasma HIV-1 RNA monitoring and associated costs that must be balanced against the high costs and potential toxicities of second-line regimens which empirically include NRTIs [25]. An intriguing association was observed between reversion of NRTI resistance mutations detectable by population sequencing at virologic failure and response to FTC/TDF intensification in ACTG A5230, perhaps suggesting that some degree of re-sensitization may occur, although longer periods of observation are needed to assess the durability of responses and the potential impact of minor variants on longer term outcomes. The continued follow-up of all patients over 2 years on ACTG A5230 will provide more information on the durability of the strategy of LPV/r monotherapy with intensification with FTC/TDF.

This study does have a number of limitations. It is a pilot, open-label uncontrolled study and the results must be cautiously interpreted. A larger randomized trial comparing second-line ART with LPV/r monotherapy followed by FTC/TDF intensification for inadequate responses vs. three-drug LPV/r-containing ART is warranted. Further, the more intensive monitoring and support provided in a clinical trial may limit generalizing to responses in clinical cohorts. Finally, at present, we are only able to report results through the primary endpoint follow-up to week 24; follow-up in ACTG A5230 is ongoing and will continue until each patient has reached 104 weeks (expected in April 2012).

In summary, persons failing first-line ART with NNRTI-containing regimens have extensive NRTI and NNRTI resistance mutations, which may carry important implications for second-line ART choices. These pilot results suggest promising early responses to the strategy of second-line LPV/r monotherapy, coupled with further virologic suppression following intensification with FTC/TDF among persons with virologic failure.

Acknowledgements

The authors wish to thank all ACTG A5230 study patients for their participation. In addition, the authors thank the Clinical Research Site members and their supporting grants at the five sites who conducted ACTG A5230 (Drs Agnes Moses and Albert Mwafongo, UNC Project Lilongwe, U01AI069518; Drs Venance Maro and John Shao, KCMC, U01069484; Professor Thira

Sirisanthana and Ms Patchanee Samutarlai, Chiang Mai University; Ms Petronilla Borain and Vuyokazi Jezile, University of Witswatersrand, U01AI38858; Ms Jabin Sharma and Dr Poongulali, YRGCARE, U01AI069432) and additional members of the ACTG A5230 Protocol Team (Heather Vezina, Amy Kambrink, Bernadette Jarocki, Vicki Bailey, A.K. Shrikrishnan and Eric Lawrence).

The project described was funded by award U01AI068636 from the National Institute for Allergy and Infectious Diseases, and was further supported by the National Institute of Mental Health and the National Institute of Dental and Craniofacial Research. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of Allergy and Infectious Diseases or the National Institutes of Health. J.A.B. is supported by NIH awards U01 AI069484, P30 AI64518, D43 TW006732, D43 CA153722 and HRSA award T84HA21123. M.R.N. is an employee of Abbott.

Funding support received from the National Institute for Allergy and Infectious Diseases of the United States National Institutes of Health, and Abbott. Drug supplies were provided by Abbott and Gilead Sciences Inc.

Conflicts of interest

There are no conflicts of interest.

References

1. Bartlett J, Shao J. **Success, challenges and limitations of antiretroviral therapy in resource-limited settings.** *Lancet Infect Dis* 2009; **9**:637–649.
2. Sungkanuparph S, Manosuthi W, Kiertiburanakul S, Piyavong B, Chumpathat N, Chantratita W. **Options for second-line antiretroviral regimens for HIV type-1 infected patients whose initial regimen of a fixed-dose combination of stavudine, lamivudine and nevirapine fails.** *Clin Infect Dis* 2007; **44**:447–452.
3. Marconi V, Sunpath H, Li 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.
4. Kumarasamy N, Madhavan V, Venkatesh K, Saravanan S, Kantor R, Balakrishnan P, *et al*. **High frequency of clinically significant mutations after first-line generic highly active antiretroviral therapy failure: Implications for second-line options in resource-limited settings.** *Clin Infect Dis* 2009; **49**:306–309.
5. Hosseinipour M, van Oosterhout J, Joep J, Weigel R, Phiri S, Kamwendo D, *et al*. **The public health approach to identify antiretroviral therapy failure: high level nucleoside reverse transcriptase resistance among Malawians failing first-line antiretroviral therapy.** *AIDS* 2009; **23**:1127–1134.
6. Wallis CL, Mellors JW, Venter F, Sanne I, Stevens W. **Varied HIV Drug resistance patterns among patients on failing antiretroviral therapy in the South African national roll-out programme.** *J Acquir Immune Defic Syndr* 2010; **53**:480–484.
7. Delfraissy J, Flandre P, Deluagerre C, Ghosn J, Horban A, Girard P, *et al*. **Lopinavir/ritonavir monotherapy or plus zidovudine and lamivudine in antiretroviral-naïve HIV-infected patients.** *AIDS* 2008; **22**:385–393.
8. Ghosn J, Flandre P, Cohen-Codar I, Girard P, Chaix M, Raffi F, *et al*. **Long-term (96 week) follow-up of antiretroviral-naïve HIV-infected patients treated with first-line lopinavir/ritonavir monotherapy in the MONARK trial.** *HIV Med* 2010; **11**:137–142.
9. Deluagerre C, Flandre P, Chaix M, Ghosn J, Raffi F, Dellamonica P, *et al*. **Protease inhibitor resistance analysis in the MONARK trial comparing first-line lopinavir-ritonavir monotherapy to lopinavir-ritonavir plus zidovudine and lamivudine triple therapy.** *Antimicrob Agents Chemother* 2009; **53**:2934–2939.
10. Chesney M, Ickovics J, Chambers D, Gifford A, Neidig J, Zwickl B, Wu A. **Self-reported adherence to antiretroviral medications among participants in HIV clinical trials: the AACTG Adherence Instruments.** *AIDS Care* 2000; **12**:255–266.
11. Rhee S-Y, Gonzales M, Kantor R, Betts B, Ravela J, Shafer R. **Human immunodeficiency virus reverse transcriptase and protease sequence database.** *Nucleic Acids Res* 2003; **31**:298–303.
12. Shafer R. **Rationale and uses of a public HIV drug-resistance database.** *J Infect Dis* 2006; **194**:S51–S58.
13. WHO Consultation on ART Failure in the Context of the Public Health Approach. www.who.int. [Accessed 25 November 2011]
14. Pujades-Rodriguez M, O'Brien D, Humblet P, Calmy A. **Second-line antiretroviral therapy in resource-limited settings: the experience of Medecins Sans Frontieres.** *AIDS* 2008; **22**:1305–1312.
15. Harries A, Zachariah R, van Oosterhout J, Reid S, Hosseinipour M, Arendt V, *et al*. **Diagnosis and management of antiretroviral-therapy failure in resource-limited settings in sub-Saharan Africa: challenges and perspectives.** *Lancet Infect Dis* 2010; **10**:60–65.
16. Keiser O, Orrell C, Egger M, Wood R, Brinkhoff M, Furrwr H, *et al*. **Public-health and individual level approaches to antiretroviral therapy: township South Africa and Switzerland compared.** *PLoS Med* 2008; **5**:e148.
17. Fox M, Ive P, Long L, Maskew M, Sanne I. **High rates of survival, immune reconstitution and virologic suppression on second-line antiretroviral therapy in South Africa.** *J Acquir Immun Def Syndr* 2010; **53**:500–506.
18. Murphy R, Sunpath H, Lu Z, Chelin N, Losia E, Gordon M, *et al*. **Outcomes after virologic failure of first-line ART in South Africa.** *AIDS* 2010; **24**:1007–1012.
19. Hosseinipour M, Kumwenda J, Weigel R, Brown L, Mzinganjira D, Mhango B, *et al*. **Second-line treatment in the Malawi antiretroviral programme: high early morality, but good outcomes in survivors, despite extensive drug resistance at baseline.** *HIV Med* 2010; **11**:510–518.
20. Pulido F, Arribas J, Delgado R, Cabrero E, Gonzalez-Garcia J, Perez-Elias M, *et al*. **Lopinavir-ritonavir monotherapy versus lopinavir-ritonavir and two nucleosides for maintenance therapy of HIV.** *AIDS* 2008; **22**:1–9.
21. Arribas J, Delgado R, Arranz A, Munoz R, Portilla J, Pasquau J, *et al*. **Lopinavir-ritonavir monotherapy versus lopinavir-ritonavir and 2 nucleosides for maintenance therapy of HIV: 96-week analysis.** *J Acquir Immune Defic Syndr* 2009; **51**:147–152.
22. Wallis CL, Mellors JW, Venter WD, Sanne I, Stevens W. **Protease inhibitor resistance is uncommon in HIV-1 subtype C infected patients on failing second-line lopinavir/r-containing antiretroviral therapy in South Africa**. *AIDS Res Treat* 2011; **(2011)**:769627.
23. Bunupuradah T, Chetchotisakd P, Ananworanich J, Munsakui W, Jirajariyavet S, Kantipong P, *et al*. **Second line lopinavir/ritonavir monotherapy was inferior to tenofovir/lamivudine//lopinavir/ritonavir in patients who failed NNRTI-regimen: HIV STAR study.** In *Abstract Presented at the Conference on Retroviruses and Opportunistic Infections*; February 2011; Boston, MA; abstract #584.
24. Pillay D, Goodall R, Gilks C, Yirrell D, Gibb D, Spyer M, *et al*. **Virologic findings from the SARA trial: boosted PI monotherapy as maintenance second-line ART in Africa.** *J Int AIDS Soc* 2010; **13**:O20.
25. Long L, Fox M, Sanne I, Rosen S. **The high cost of second-line antiretroviral therapy for HIV/AIDS in South Africa.** *AIDS* 2010; **24**:915–919.