Antiretroviral Drug Resistance in HIV-1–Infected Patients Experiencing Persistent Low-Level Viremia During First-Line Therapy

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Population sequencing was performed for persons identified with persistent low-level viremia in 2 clinical trials. Persistent low-level viremia (defined as plasma HIV-1 RNA level >50 and <1000 copies/mL in at least 2 determinations over a 24-week period, after at least 24 weeks of antiretroviral therapy) was observed in 65 (5.6%) of 1158 patients at risk. New resistance mutations were detected during persistent low-level viremia in 37% of the 54 evaluable cases. The most common mutations were M184I/V (14 cases), K103N (9), and M230L (3). Detection of new mutations was associated with higher HIV-1 RNA levels during persistent low-level viremia.

Received 12 January 2011; accepted 31 March 2011.

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Potential conflicts of interest: B. T. has served on the advisory board and/or received research funding to Northwestern University from Tibotec, ViiV, and Monogram. R. H. has received honoraria or consultant fees from Abbott, Bristol-Myers Squibb, Gilead Sciences, GSK, KKC, Merck, Monogram, Pfizer, Quidel, Roche, and Tibotec, and has received research support (to UCSD) from Abbott, GlaxoSmithKline, Pfizer, and Merck. D. R. K. is a consultant to and/or has received research funding from Abbott, Boehringer-Ingelheim, Gilead Sciences, GlaxoSmithKline, Merck, Roche, and ViiV. J. J. E. is a consultant to Abbott, Bristol-Myers Squibb, GlaxoSmithKline, Merck, ViiV, and Tibotec, and has received research support (to UNC) from GlaxoSmithKline and Merck. H. J. R. has served on an advisory panel for Roche. All other authors. no conflicts.

Presented in part: International HIV and Hepatitis Virus Drug Resistance Workshop and Curative Strategies, Dubrovnik, Croatia, 8–12 June 2010. Abstract 30.

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The Journal of Infectious Diseases 2011;204:515–20

0022-1899 (print)/1537-6613 (online)/2011/2044-0006$14.00
DOI: 10.1093/infdis/jir353

Persistent low-level viremia is associated with higher immune activation [1], increased risk of virologic failure [1, 2], and perhaps, increased mortality [3], compared with plasma HIV-1 RNA level <50 copies/mL. Antiretroviral drug resistance during low-level viremia is difficult to study because of the limitations of conventional genotype testing. Studies of antiretroviral resistance during low-level viremia have rarely addressed patients receiving first-line antiretroviral therapy (ART) [4–8]. The present study aimed to describe new resistance mutations in patients with low-level viremia during first-line ART and to evaluate the risk factors and virologic plus immunologic consequences.

METHODS

Participants were identified retrospectively from 2 AIDS Clinical Trials Group clinical trials: (1) all arms of A5142, which included lopinavir-ritonavir plus efavirenz, lopinavir-ritonavir plus 2 nucleos(t)ide reverse transcriptase inhibitors (NRTI), and efavirenz plus 2 NRTIs [9], and (2) efavirenz-containing arms of A5095, which included efavirenz plus lamivudine-zidovudine or lamivudine-zidovudine-abacavir [10]. The NRTIs in A5142 were lamivudine plus zidovudine, stavudine, or tenofovir. The present study was conducted with informed consent obtained for A5142 and A5095.

Low-level viremia was defined as viral load (VL) >50 and <1000 copies/mL in at least 2 determinations over a 24-week period, after at least 24 weeks of ART. This definition included participants who did not achieve VL <50 copies/mL by week 24 and those who achieved VL <50 copies/mL but subsequently had viral rebound. The end of low-level viremia was defined as the first VL ≤50 or ≥1000 copies/mL, unless it occurred within a 6-month period during which at least 2 other VL determinations met the low-level viremia definition (this was to account for potential VL measurement error).

VL was determined using the ultrasensitive Roche Amplicor HIV-1 Monitor assay (version 1.0 and/or 1.5). The study definition of low-level viremia falls within the approved range of this assay. Pretreatment reverse transcriptase (RT) and protease (PR) sequences were obtained from the parent study, or population sequencing was performed on stored pretreatment plasma samples when sequence data were not available. To detect mutations during low-level viremia, the last plasma sample obtained during low-level viremia was sequenced for all participants. If any mutation was detected, the first sample obtained during low-level viremia was sequenced; if there was a discordance between the first and last samples, all the samples

obtained during low-level viremia for that participant were sequenced. To enhance the sensitivity for detecting mutations at low VL, ultracentrifugation of 1.5 mL of stored plasma was performed to concentrate viral RNA (28,100 $\times g$ for 2 hours at 4°C) before RNA extraction (QIAamp viral RNA minikit; QIAGEN). HIV-1 PR (codons 1–99, HXB2 nucleotides 2254–2549) and RT (codons 1–234, HXB2 nucleotides 2550–3249) were reverse transcribed and amplified in a coupled reverse transcription polymerase chain reaction (PCR; 40 cycles), followed by a 40-cycle nested-PCR using gene-specific primers. Population sequencing was performed on resulting purified amplicons with use of the Applied Biosystems Taq Dye Deoxy Terminator cycle sequencing kits, which use a fluorescently labeled dideoxy-nucleotide chain termination method, and resolved on an ABI 3730 automated DNA sequencer (Applied Biosystems). Sequence processing was done using the Sequencher program (Genecodes). Sequences were aligned to the HIV-1 subtype B reference strain HXB2 (GenBank accession no. K03455). Each sample was amplified in duplicate, and the amplicons were pooled for analysis. Phylogenetic analysis using PhyML was used to confirm sequence identity and to exclude PCR contamination.

The mutations of interest were any RT or major PR mutation according to the International AIDS Society–USA [11]. Analyses considered all mutations identified before treatment and during low-level viremia. Risk factors for developing low-level viremia were explored using Cox proportional hazards, including all patients from A5142 and A5095 with at least 48 weeks of follow-up; follow-up was censored at initial treatment discontinuation. The assumption of proportional hazards with respect to pretreatment covariates was evaluated with interaction terms with the natural logarithm of time. Among patients with genotype available before treatment and during low-level viremia, the distributions of candidate risk factors were compared between participants with new resistance mutations and those without new resistance mutations with use of Wilcoxon rank sum and Fisher's exact tests; adjusted associations were examined using logistic regression with exact methods as feasible. Sensitivity analyses were conducted that excluded participants with single VL outliers ≥1000 copies/mL during low-level viremia. The immunologic consequences were evaluated using a nested case-control study in which each low-level viremia case was frequency matched with at least 1 control on the basis of pretreatment VL (<100,000 or ≥100,000 copies/mL), pretreatment $CD4^+$ T-cell count (<200 or ≥200 cells/mm^3), randomized regimen, study, and race/ethnicity. Evaluation of changes in immunologic parameters for case patients and control subjects used a paired analysis (Wilcoxon signed rank test) in which the observed change during low-level viremia for each case patient was compared with the mean change during the same period in their set of matched control subjects.

RESULTS

Population With Low-level Viremia

Sixty-five (5.6%) of 1158 participants receiving initial ART in A5142 (39 [7%] of 562) and A5095 (26 [4%] of 596) experienced low-level viremia. These 65 participants received 2 NRTIs plus efavirenz (31%), 2 NRTIs plus lopinavir-ritonavir (31%), lopinavir-ritonavir plus efavirenz (22%), and 3 NRTIs plus efavirenz (17%). Their median pretreatment VL was 5.1 $\log_{10}$ copies/mL, and median CD4 cell count was 121 cells/mm^3. Median time from treatment initiation to onset of low-level viremia was 39 weeks (range, 25–119 weeks); median duration was 30 weeks (range, 23–93 weeks). Forty-two participants (68%) achieved VL <50 copies/mL before onset of low-level viremia. The median number of samples during low-level viremia was 5 (range, 3–12).

Participants receiving 2 NRTIs plus lopinavir-ritonavir had an estimated 2.7-fold greater hazard of low-level viremia (95% confidence interval [CI], 1.4–5-fold), compared with participants receiving 2 NRTIs plus efavirenz. Pretreatment VL ≥6 $\log_{10}$ copies/mL was associated with a 2.2-fold increased risk of low-level viremia (95% CI, 1–4.6-fold). Each 50 cells/mm^3 increase in pretreatment CD4 cell count was associated with a 10% lower hazard of low-level viremia (hazard ratio, .9; 95% CI, 0.8–1.0). Associations with sex, age, race/ethnicity, and time-updated recent adherence [12] were not detected ($P > .15$); no violation of the proportional hazards assumption was detected ($P > .6$).

New Resistance Mutations During Low-Level Viremia

Resistance data were available for 59 (91%) of 65 patients with low-level viremia: 37 (95%) of 39 from A5142 and 22 (85%) of 26 from A5095. No plasma sample was available for 4 patients; 2 samples failed to amplify. Four participants had pretreatment mutations in RT (K103N, V106I, G190A, T215Y; V90I, V179D/V; V179D; and V108I) and 1 in PR (M46I/M). Resistance data were available before treatment and during low-level viremia for 54 participants. New resistance mutations were detected during low-level viremia in 20 (37%) of these participants (Table 1), all in the RT region except D30D/N in one participant. The most common mutations were M184I/V (14 cases), K103N (9), and M230L (3). Fourteen participants had new resistance mutations in the first sample during low-level viremia; of these, 3 subsequently accumulated additional mutations.

Among 39 participants who continued to receive their initial regimen after low-level viremia, 65% of those with evidence and 74% of those without evidence of new resistance subsequently achieved VL <50 copies/mL at least once ($P = .36$). Two consecutive VL >1000 copies/mL after low-level viremia occurred more frequently in participants with new mutations (30% vs 9%; $P = .05$).

Table 1. Characteristics of the Patients New Resistance Associated Mutations (RAM) Detection During Persistent Low-Level Viremia

Patid	ACTG trial	ART regimen	Pretreatment CD4 count (cells/mm^3)	Pretreatment VL (log_{10} copies/mL	Pretreatment RAM	RAM during low-level viremia	VL at time of RAM detection (copies/mL)[a]	Week of treatment when RAM was detected[b]	Follow-up VL after low-level viremia while on initial treatment[c]
1	A5095	2NRTI + EFV	327	3.2	**V**90I, **V**179D/V	**M**184V **M**230L**/**M	112	33	VF
2	A5142	2NRTI + LPV	636	4.7	—	**M**184V	<50*	67	VF
3	A5095	2NRTI + EFV	37	4.8	—	**K**101E, **K**103N, **M**184V M230L	76	58	VL <50 copies/mL
4	A5095	2NRTI + EFV	373	4.6	K103N, V106I, G190A T215Y	**M**184V (A62A**/**V)	101	32	Off treatment right after **low-level viremia**
5	A5142	2NRTI + LPV	6	4.9	—	**V**106M**	120	32	VL <50 copies/mL and then VF
6	A5142	LPV/EFV	152	4.9	—	**K**103N, **M**230L	105	72	VL <50 copies/mL
7	A5142	2NRTI+EFV	192	5.3	—	**V**106I	159	32	VL <50 copies/mL
8	A5095	2NRTI+EFV	71	6.4	—	**M**184V	8,322***	144	Off treatment right after **low-level viremia**
9	A5095	2NRTI+EFV	201	4.2	—	**Y**188C (**D**67D/N)	203	96	VF
10	A5142	2NRTI+EFV	14	5.9	—	**K**70K/R	105	33	Off treatment right after **low-level viremia**
11	A5095	2NRTI+EFV	304	4.2	—	**K**103N, **M**184V, **G**190A	494	26	**One VL >1000 copies/mL and one VL <50 copies/mL**
12	A5095	3NRTI+EFV	284	4.1	—	**L**74V, **K**103N, **Y**115F, **M**184V	368	111	Off treatment right after **low-level viremia**
13	A5095	3NRTI+EFV	16	5.0	—	**K**103N, **M**184V, **P**225H**/**P	238	32	**One VL <50 copies/mL, then low-level viremia range**
14	A5095	2NRTI+EFV	267	4.6	—	**K**103N, **M**184V (**V**108I)	460	82	VF
15	A5142	2NRTI+LPV	74	4.8	—	**V**75I	253	64	VL <50 copies/mL*****
16	A5142	2NRTI+LPV	88	5.8	—	**M**184V	362	80	No information available
17	A5095	3NRTI+EFV	52	6.0	—	**K**103N, **M**184V (**P**225H)	417	48	VF*****
18	A5095	3NRTI+EFV	27	5.5	—	**K**103N (**M**184V)	531	73	VF
19	A5142	2NRTI+LPV	212	4.2	—	**M** 184V (**D**30D/N****)	383	49	Off treatment right after **low-level viremia**
20	A5142	LPV/EFV	216	5.3	—	**Y**181C/Y	57	31	VL <50 copies/mL

NOTE. [a] First occurrence of RAM during **low-level viremia** (RAM that emerged later).
[b] Based on two consecutive measurements unless otherwise noted - VF: virologic failure (VL > 1,000 copies/mL).
[c] **Time between randomized treatment initiation and collection of sample during low-level viremia.**
* This sample was sequenced because M184V was detected in the last sample obtained during low-level viremia, but was not present in the first **low-level viremia** sample from this patient.
** No known exposure to an NNRTI.
*** VL measurements (copies/mL) in the 6 months around this measurement were 59, 57, 114, 8,322, and 963.
**** Only new resistance mutation in the protease gene.
***** Based on single HIV-1 RNA level after **low-level viremia**.

Factors Associated With New Mutations

Table 2 shows the distributions of candidate risk factors for particpants with versus those without new mutations. VL during low-level viremia was the primary factor associated with new resistance. Participants in whom new mutations were detected tended to have a higher VL at the start of low-level viremia ($P = .03$) and higher minimum ($P < .001$), maximum ($P < .001$), and mean VL during low-level viremia ($P < .001$). There was also a suggestion that pretreatment VL was lower in those with new resistance (median, 4.84 $\log_{10}$ vs 5.17 $\log_{10}$ copies/mL; $P = .09$). Similar results were seen in sensitivity analyses. New mutations were detected in 0 (0%) of 10 participants with maximum VL 51–100 copies/mL, compared with 5 (38%) of 13 with maximum VL 101–200 copies/mL and

Table 2. Association of Patient Parameters With New Resistance Detection During Low-Level Viremia (Includes Only Patients With Genotype Available Pretreatment and During Low-Level Viremia)

		Total (N = 54)	No new resistance (N = 34)	New resistance (N = 20)	*P* value
Age (years)	Median	38	38.5	37.5	.40*
	10%, 90%	31, 50	31, 50	26.0, 47.5	
Sex	Male	46 (85%)	30 (88%)	16 (80%)	.45**
	Female	8 (15%)	4 (12%)	4 (20%)	
Race/ethnicity	White non-Hispanic	18 (33%)	13 (38%)	5 (25%)	.007**
	Black non-Hispanic	21 (39%)	8 (24%)	13 (65%)	
	Hispanic (regardless of race)	15 (28%)	13 (38%)	2 (10%)	
Pretreatment VL ($\log_{10}$ copies/mL)	Median	5.07	5.17	4.84	.09*
	10%, 90%	4.22, 6.05	4.52, 6.05	4.13, 5.98	
Pretreatment CD4 count (cells/mm^3)	Median	126	121	172	.44*
	10%, 90%	16, 327	22, 295	15, 350	
Length of **low-level viremia** (weeks)	Median	32	33	27	.19*
	10%, 90%	23, 56	24, 56	23, 45	
First week of **low-level viremia** (week on study)	Median	40	33	49	.19*
	10%, 90%	31, 97	30, 97	32, 104	
Number of outliers ≤50 copies/mL in **low-level viremia**	0	26 (48%)	11 (32%)	15 (75%)	.006**
	1	26 (48%)	21 (62%)	5 (25%)	
	2	2 (4%)	2 (6%)	0 (0%)	
Number of outliers ≥1000 copies/mL in **low-level viremia**	0	45 (83%)	32 (94%)	13 (65%)	.009**
	1	9 (17%)	2 (6%)	7 (35%)	
ART discontinuation > 7 days during **low-level viremia**	No	50 (93%)	32 (94%)	18 (90%)	.62**
	Yes	4 (7%)	2 (6%)	2 (10%)	
Adherence during **low-level viremia**	<100%	18 (33%)	9 (26%)	9 (45%)	.23**
	=100%	32 (59%)	23 (68%)	9 (45%)	
	Unknown	4 (7%)	2 (6%)	2 (10%)	
First VL during **low-level viremia** (copies/mL)	Median	113	90	181	.032*
	10%, 90%	59, 383	58, 297	67, 400	
Minimum VL during **low-level viremia** (copies/mL)	Median	38	25	71	<.001*
	10%, 90%	25, 153	25, 72	25, 256	
Maximum VL during **low-level viremia** (copies/mL)	Median	282	150	615	.001*
	10%, 90%	86, 1,476	80, 592	128, 7,589	
Time adjusted area under the curve (copies/mL)	Median	80	68	143	<.001*
	10%, 90%	53, 480	52, 137	69, 931	

NOTE. *Exact Wilcoxon Test.
**Fisher's Exact Test.

15 (48%) of 31 with maximum VL >200 copies/mL. Attainment of VL ≤50 copies/mL at any time during low-level viremia was negatively associated with new mutations ($P = .006$), whereas a VL increase to ≥1000 copies/mL was positively associated ($P = .009$).

A larger proportion of participants with new mutations were black, non-Hispanic (65% of participants with vs 24% of those without new mutations; odds ratio, 4.2; 95% CI, 1.1–16.4). Adjustment for maximum VL during low-level viremia attenuated this association, as did adjustment for mean VL. No associations between detection of new mutation and age, sex, pretreatment CD4 cell count, attainment of VL <50 copies/mL before low-level viremia, or nonadherence before low-level viremia were observed ($P > .1$).

Immunologic Consequences of Low-Level Viremia

No difference in CD4 cell count change over the low-level viremia period between 64 case patients and the 64 sets of matched control subjects was apparent (median difference, −16 cells/mm^3; interquartile range [IQR], −79 to 49 cells/mm^3; $P = .29$). There were smaller changes in CD4 cell percentage (median difference, −0.8; IQR, −3.7 to 1.3; $P = .016$) and in CD4:CD8 ratio (median relative difference, 0.93; IQR, 0.73–1.06; $P < .001$) in patients with low-level viremia.

DISCUSSION

This study explored new resistance mutations in patients with low-level viremia during initial ART. Approximately 6% of patients who initiated lopinavir-ritonavir– or efavirenz-containing ART developed low-level viremia. Pretreatment VL ≥6 $\log_{10}$ copies/mL, lower pretreatment CD4 cell count, and treatment with lopinavir-ritonavir were risk factors for low-level viremia. The association between lopinavir-ritonavir and low-level viremia as defined in this study is consistent with previously observed slower viral suppression to <50 copies/mL with lopinavir-ritonavir–based regimens, compared with efavirenz-based regimens [9]. New resistance mutations were detected during low-level viremia in 37% of participants. Higher levels of viremia during low-level viremia but not higher pretreatment VL were associated with increased risk of new mutations.

The association found between higher levels of viremia during low-level viremia and increased risk of new resistance was consistent when considering maximum, minimum, or mean VL. New mutations were detected in some patients with maximum observed VL <200 copies/mL during low-level viremia, an important observation considering recent guidelines that virologic failure in clinical practice can be defined as VL >200 copies/mL [12]. Patients with isolated increases in VL to >1000 copies/mL were the most likely to have evidence of new resistance. Participants with low-level viremia and new mutations appeared to be at a higher risk for subsequent virologic failure, but some patients with new mutations achieved viral suppression without a change in regimen. Higher levels of nonadherence and viremia not captured in the study possibly influenced these findings. Different results might be obtained using newer real-time PCR methods for VL measurement.

Mutations associated with resistance to lamivudine-emtricitabine (M184V/I) and efavirenz-nevirapine (K103N) were most frequently detected. The detection of etravirine resistance–associated mutations in some patients receiving efavirenz suggests that etravirine may not be fully active in subsequent regimens. The only major protease inhibitor (PI) mutation detected was the D30D/N. This participant was receiving lopinavir-ritonavir and had no known exposure to nelfinavir. The mutation may represent a transmitted variant not detected before treatment. Ritonavir-boosted PIs appear to be likely to retain full activity in patients with low-level viremia while receiving boosted PI-based first-line ART. Thymidine analogue mutations D67D/N and K70R were present in 2 patients receiving thymidine analogues. Accordingly, thymidine analogue activity may become occasionally compromised during low-level viremia. The L74V mutation was detected in a patient receiving abacavir.

New mutations during low-level viremia appeared to be more common in black (non-Hispanic) patients, but this finding should be considered cautiously because of the small number of events and the confounding effect of VL. An association between African-American ethnicity and resistance during low-level viremia has been reported previously, although in a treatment-experienced population [5]. If confirmed, possible explanations could include differential adherence [13] or race-based genetic factors that may influence drug metabolism and plasma concentrations [14].

In conclusion, new resistance mutations were detected in patients experiencing low-level viremia during first-line ART. Those with higher VL during low-level viremia had a greater risk, but this association had limited precision. Techniques for detecting resistance during low-level viremia should be validated for clinical use, and the clinical consequences of low-level viremia and mutations detected during low-level viremia should be investigated further.

Funding

This work was supported by the "Agence Nationale de Recherche sur le SIDA" Fellowship Grant for Post-Graduate Studies (to S. G.),

National Institutes of Health (K24-AI064086 to R.H.), UCSD Center for AIDS Research (AI36214 to R. H.), UCSD ACTU (AI69432 to R. H.),

National Institutes of Health (K24 RR061482 to D. K.), ACTG Virology Support Laboratory (U01 AI 068636 to D. K.),

ACTU (AI69423 to J. J. E.), UNC Center for AIDS Research (AI50410 to J. J. E.), CTSA (RR025747 to J. J. E.), and

Statistical and Data Management Center of the AIDS Clinical Trials Group, under the National Institute of Allergy and Infectious Diseases (1 U01 AI068634 to H. J. R. and E. A.)

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