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Short Communication

Reversal Periods and Patterns from Drug-Resistant to Wild-Type HIV Type 1 after Cessation of Anti-HIV Therapy

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ABSTRACT

Anti-HIV drug-resistant virus reverts to wild type following discontinuation of antiretroviral therapy (ART). This study aimed to determine the reversal period. ART was discontinued in 16 patients harboring drug-resistant viruses. Resistant mutations of reverse transcriptase (RT) and protease (PR) genes of plasma- and peripheral blood mononuclear cells (PBMC)-derived viruses were examined by direct sequencing monthly until the disappearance of mutants (median follow-up period: 8.9 months). Only wild-type virus was detected in 50% of patients at 6.3 months (quartiles, 3.2–20.7 months) and at 9.2 months (quartiles, 5.7–13.8 months) in plasma- and PBMC-derived viruses, respectively, after ART interruption. Among the 133 resistance-associated mutations identified at ART interruption, half the RT and PR mutations shifted to wild type in 3.2 months in plasma, 6.7 months of RT, and 5.7 months of PR in PBMC, respectively. In plasma- and PBMC-derived viruses, the PR mutations reverted earlier than the RT mutations. These results could be relevant as to when to perform drug-resistance testing.

THE EMERGENCE OF DRUG-RESISTANCE-ASSOCIATED MUTATIONS leads to treatment failure and may limit future treatment options. Therefore, inclusion of drug-resistance testing is recommended in anti-HIV-1 treatment guidelines, especially after failure of standard regimens.[1,2] A number of studies showed that drug-resistance testing improved the benefits of antiretroviral therapy (ART).[3–8] For drug resistance testing, plasma and peripheral blood mononuclear cell (PBMC) can be used as clinical specimens.[9] Using direct sequencing, we reported previously the earlier detection of resistant mutations in plasma than in PBMC.[10] Accordingly, we recommended the use of plasma for early detection of drug resistance during therapy in those patients who fail to respond to antiretroviral treatment. Clinically, even when patients develop virologic failure [rebound of plasma HIV-1 viral load (VL)], the CD4 count remains sufficiently high for treatment interruption, at least in some patients. In such cases, the timing of genotypic drug resistance testing is of practical importance. Discontinuation of treatment causes the reversion of resistance mutations to wild-type viruses.[11–18] Previous studies indicated that resistance mutations of plasma viruses could rapidly become undetectable either partially or entirely from 14 days to 4 months after ART cessation.[12–18] The reversion of mutations to wild type is considered to be due to the low replication fitness of mutant variants and outgrowth of wild type viruses when the drug-selective pressure is withdrawn.[17,21–22] However, the time course and pattern of this reversion have not been studied in detail in heavily treated patients. Clarification of this issue will help determine the most appropriate time and sample for performing genotypic-resistance testing after ART cessation.

The study subjects were 16 HIV-1-infected patients who had been known to have drug-resistance virus beforehand and discontinued antiretroviral therapy from August 1998 through December 2002 for a variety of reasons. All patients regularly consulted the AIDS Clinical Center at the International Medical Center of Japan, Tokyo, and gave written informed consent. Their demographic data and clinical characteristics at the time of quitting ART are listed in Table 1. Their blood samples were

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TABLE 1. DEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF PATIENTS[a]

Pt	*Sex*	*Age (years)*	*Risk factor*	*CD4 cells/μl*	*log_{10} VL month 0*	*log_{10} VL month 1*	*Duration of ART (months)*	*Drugs ever used*	*Reasons of ART stop*
1	M	40	Bisexual	404	5.2	5.3	70.4	AZT, 3TC, d4T, ddI, NFV, RTV, SQH	Virological failure
2	M	23	Hemophilia	103	5	5	46.6	ddC, d4T, 3TC, AZT, ddI, SQH, NFV, IDV	Virological failure
3	M	36	MSM	209	3.5	3.5	91.8	AZT, 3TC, d4T, ddI, NFV	Virological failure
4	M	22	Hemophilia	116	5.1	4.3	71.9	ddC, d4T, 3TC, ABC, ddI, AZT, EFV, SQH, NFV, APV	Virological failure
5	M	26	Hemophilia	30	5.7	5.2	50.2	d4T, 3TC, ddI, AZT, NFV, IDV, RTV	Virological failure
6	M	28	Hemophilia	93	3.6	3.7	129.7	d4T, ddI, AZT, 3TC, ABC, ddC, EFV, RTV, SQH, IDV, NFV, APV	Virological failure
7	M	29	Hemophilia	698	1.7	5	108.6	AZT, 3TC, d4T, ddI, ABC, EFV, RTV, SQH	Side effects
8	M	24	Hemophilia	35	5.1	5.1	67	AZT, 3TC, ddC, SQH, RTV	Virological failure
9	F	42	Heterosexual	690	1.8	4.4	90	AZT, ddI, d4T, 3TC, ABC, EFV, SQH, APV, NFV	Poor adherence
10	M	19	Hemophilia	586	1.8	4.2	107.6	AZT, ddC, d4T, 3TC, ddI, ABC EFV, IDV, RTV, NFV, SQH, LPV/r	Poor adherence
11	M	24	Hemophilia	644	1.7	4.5	117.6	AZT, ddI, 3TC, IDV, RTV	Poor adherence
12	M	22	Hemophilia	138	4.5	4.5	53.4	AZT, ddC, d4T, 3TC, ddI, NVP, SQH, RTV, IDV	Virological failure
13	M	34	Bisexual	276	1.7	3.6	47.6	d4T, 3TC, ddI, ABC, AZT, NFV, IDV, RTV, LPV/r	Poor adherence
14	M	42	MSM	420	4.3	5.3	49.8	AZT, 3TC, d4T, ddI, IDV, RTV, SQH	Virological failure
15	M	39	Hemophilia	544	1.7	4.5	140.8	AZT, d4T, 3TC, NVP, NFV	Side effects
16	M	37	Bisexual	525	4.4	4.6	69.9	AZT, ddC	Virological failure
Mean		30.4		344	3.5	4.5	82		

[a]M, male; F, female; MSM, men having sex with men; VL, HIV-1 viral load in plasma; month 0, time when ART was stopped; month 1, 1 month after ART was stopped. AZT, zidovudine; 3TC, lamivudine; d4T, stavudine; ddI, didanosine; ddC, zalcitabine; ABC, abacavir; EFV, efavirenz; NVP, nevirapine; NFV, nelfinavir; RTV, ritonavir; SQH, saquinavir hard gel capsule; DOV, indinavir; APV, amprenavir; LPV/r, lopinavir + ritonavir.

collected monthly. Measurements of VL (Amplicor HIV-Monitor, Roche Molecular Systems, Inc., NJ) and CD4 and CD8 lymphocyte counts (monoclonal antibodies and flow cytometry) were performed at each blood sampling.

PBMC were separated by centrifugation from 7 ml EDTA-treated blood. PBMC and plasma were stored at −80°C until sequence analysis. The method of sequence analysis was reported previously.[10] Briefly, total RNA was extracted from 100 μl plasma and DNA was extracted from 1×10^6 PBMCs (SMITEST Ex R&D Kit, Japan). The RNA sample was subjected to reverse transcription (RT) followed by nested polymerase chain reaction (PCR) using primers targeting the RT gene and protease (PR) gene, respectively. A DNA sample was also subjected to nested PCR using the same primers for the same targets. The primers covered 1–100 base pairs of PR and 40–240 base pairs of RT. Sequences of primer sets were published elsewhere.[10] Direct sequencing was performed on a 3730 DNA Analyzer (Applied Biosystems). A heterozygous base sequence was identified when the electrogram showed a minor peak at >50% of the major peak. The amino acid sequence was deduced with the GENETYX-WIN version 4.1 (Software Development, Tokyo) and the amino acid substitutions related to drug resistance were estimated from published data.[2] The clade of HIV-1 was determined by the sequences of RT and PR genes.

The reversal period was defined as the time interval between the date of ART interruption and the date of the disappearance of mutations confirmed by direct sequencing. When mutations (all minor mutations, in some patients) did not revert, the reversal period was defined as the date ART stopped to the date most mutations shifted to the wild-type amino acid sequence (for example, see Table 2; protease residues of plasma virus at month 5.9 of patient 2). As all HIV-1s amplified in this study were HIV-1 clade B, we regarded L63P as the polymorphism. The major mutant residues included M41L, A62V, D67N, K70R, L74V, M184V, G190S, L210W, T215F/Y, and K219E/Q of RT mutations and D30N, L33F, M46I, G48V, V82A/F, I84V, and L90M of PR mutations.[2] The follow-up period was the time interval from when ART was interrupted to when the resistance mutations disappeared.

A Kaplan–Meier survival curve was used to estimate the continuous periods of resistance mutations. The Mann–Whitney *U* test was used for group comparisons, the Wilcoxon signed rank test was used for paired comparison of the reversal period, the paired *t*-test was used for changes in CD4 count and HIV-1 viral load, and correlation analysis was used for the relationship between the reversal period and baseline CD4 count or baseline viral load, respectively. StatView version 5 was used for analysis and a p value less than 5% was considered statistically significant.

As shown in Table 1, most patients enrolled in this study had been treated over a long period of time [mean ART period: 82 months (SD, 31.6; range, 46.6–140.8 months)]. The reasons for discontinuation of ART were virologic failure in 10 cases, poor adherence in 4 cases, and side effects in 2 cases. The median follow-up period was 8.9 months (range: 2–25 months) and all patients provided blood samples for testing. None of the patients received any ART during the follow-up period. CD4 counts of 10 patients were more than 200/μl at the time of ART discontinuation. After withdrawal of ART, the CD4 count decreased a mean value of 66/μl 1 month later and continued to decrease until the disappearance of resistant mutations. The VL of 6 patients (patients 7, 9, 10, 11, 13, and 15) who discontinued ART because of side effects or poor adherence ranged from <50 to 650 copies/ml at the time of ART cessation. The VL of these patients rebounded to a mean of 4.2 $\log_{10}$ copies/ml 1 month later (designated as rebounded virus) but showed a plateau level thereafter. The VL of the other 10 patients who discontinued ART for virologic failure was stable after ART cessation.

In all 16 patients, a total of 133 resistance mutation residues with 59 RT and 74 PR were found in plasma and PBMC. The concordance of mutant residues between plasma and PBMC was 96.2% (RT mutations 93.2%, PR mutations 98.6%). All 16 patients possessed RT resistance mutations but 4 of them had no PR mutations (Table 2). In PR, both plasma and PBMC-derived viruses had 26 major resistance and 48 minor resistance residues. In contrast in RT, 52 and 50 major RT residues and 7 and 9 minor RT residues were detected in plasma and PBMC, respectively. The results showed that the resistance mutations could shift to wild type after 1 month or could persist for as long as 22 months after treatment stopped. Interestingly, in 6 patients with viral load rebound, the rebounded viruses in 5 patients (patients 7, 10, 11, 13, and 15) had the same resistant mutations as their predecessor viruses 1 month after ART cessation and then reverted to wild type thereafter. In patient 9, the rebounded virus was a wild-type virus.

As shown in Fig. 1A, after ART interruption, only wild-type virus was detected in 50% of patients at 6.3 months (quartiles, 3.2–20.7 months) and at 9.2 months (quartiles, 5.7–13.8 months) in plasma- and PBMC-derived viruses, respectively. In Fig. 1B, the reversion of 133 resistance mutations is shown by a Kaplan–Meier survival curve. Fifty percent of both PR and RT resistance mutations shifted to wild type in 3.2 months in plasma (quartiles, 1.5–3.7 months for PR, 2–10 months for RT). However, in PBMC, 50% of PR and RT mutations disappeared in 5.7 (quartile, 3.2–6.7 months) and 6.7 (quartile, 3.5–12 months) months, respectively. The reversal period of PR and RT mutations in plasma was 2.5 and 3.5 months, respectively, less than that in PBMC (both $p < 0.05$). Furthermore, the PR mutations shifted to wild type much more rapidly than RT mutations in both plasma and PBMC, although the half life of both mutation residues were the same in plasma (Wilcoxon test $p < 0.05$). In terms of the reversal period of major and minor mutations, there were no difference between them both in the PR and RT regions of plasma- or PBMC-derived viruses. There were no relationships found between the reversal periods of RT and PR mutations and the baseline CD4 cell count, baseline VL, and changes in these two surrogate markers 1 month later (data not shown).

Figure 2 shows how the mutation residues disappeared after ART cessation. We roughly divided the reversal process into two patterns. The first pattern was that resistant mutations persisted for some time and then disappeared abruptly (Fig. 2A). Most PR mutations of plasma viruses, 50% of PR mutations of provirus, and 50% of RT mutations in both types of specimens showed this pattern. The second pattern was that of a gradual decrease of mutations followed by their disappearance or persistence (Fig. 2B). One-third of RT mutations showed this pattern. Overall, all major mutations of RT and PR genes disappeared in all patients after withdrawal of ART. In contrast, the minor mutations did not disappear in some patients.

TABLE 2. RESISTANCE MUTATIONS AND REVERSAL PERIOD IN PLASMA AND PBMC AFTER ART CESSATION

Pt	*Sample*	*Months after ART cessation*	*Reverse transcriptase residues*	*Months after ART cessation*	*Protease residues*
1	Plasma	0	41L, 69D, 118I, 210W, 215Y	0	10I, 30N, 33F, 71T, 84I, 88D, 90M
		3.2	—[a]	3.2	—
	PBMC	0	41L, 69D, 118I, 210W, 215Y	0	10I/L, 30N/D, 33F/L, 71T/A, 84I/V, 88D/N, 90M
		3.2	—	3.2	—
2	Plasma	0	41L, 67N, 69D, 118I, 210W, 215Y	0	10I, 20M, 36I, 48V, 54V, 82A
		15.2[b]	41L, 210W/R	5.9	10F, 36I
	PBMC	0	41L, 67N, 69D, 118I, 210W, 215Y	0	10I, 20M, 36I, 48V, 54V, 82A
		15.2[b]	41L, 118I, 215Y	9	10F, 36I
3	Plasma	0	41L, 44D, 184V, 215Y	0	30N, 71V, 77I, 88D
		7	—	4.6	—
	PBMC	0	41L, 44D, 184V, 215Y	0	30N, 71V, 77I, 88D
		8.6	—	4.6	—
4	Plasma	0	41L, 74V, 184V, 215Y	0	10I, 20I/M, 71V, 73S, 84V, 90M
		2.8	—	4.8	—
	PBMC	0	41L, 184V, 215Y	0	10I/L, 20I, 71V/A, 73S/G, 84V/I, 90M
		4.8	—	4.8	—
5	Plasma	0	41L, 44D, 67N, 210W, 215Y	0	10I, 46I, 71T, 73S, 77I, 82F, 90M
		7.9	—	3.3	10I, 77I
	PBMC	0	41L, 44D, 67N, 184V, 210W, 215Y	0	10I, 46I, 71V, 73S, 77I, 82F, 90M
		12.5	—	6.7	10I, 77I
6	Plasma	0	41L, 74V, 184V, 215Y	0	10I, 46I, 54L, 71V, 77I, 84V, 90M
		6.3	—	1.4	77I
	PBMC	0	41L, 74V/L, V118I, 184V/M, 190G/S, 210W, 215Y	0	10I, 20M/K, 46I, 54L/I, 71V, 77I, 84V, 90M
		11.3	—	3.2	77I
7	Plasma	0	67N, 70R, 184V, 219Q	0	N[c]
		1	67N/D, 70R, 184V/M, 219Q/K		
		3.2	—		
	PBMC	0	67N, 70R, 184V, 219Q	0	N
		9.2	—		
8	Plasma	0	41L, 184V, 215F	0	20R, 36I, 54V, 71V, 82A, 90M
		5.7	—	3.7	—
	PBMC	0	41L, 184V, 215F	0	20R, 36I, 54V, 71V, 82A, 90M
		5.7	—	5.7	20R,
9	Plasma	0	41L, 67N, 70R, 215F, 219E	0	10L/I, 36I, 73S, 77I, 90M
		1	—	1	—
	PBMC	0	67N, 184M/N, 210W, 219E	0	10L/I, 71T/I, 73S, 77I, 90M
		2	—	1	—

10	Plasma	0	41L, 67N, 215F, 219Q	0	10I, 36I, 46I, 53L, 71V, 84V, 90M
		1	41L, 67N, 215F, 219Q		
		8.5[d]	219Q/K	3.7	10I, 36I
	PBMC	0	41L/M, 67N, 70R, 118V/I, 184V/M, 215F, 219Q	0	10I, 36I, 46I, 53L/F, 71V, 84V, 90M
		8.5[d]	67N, 70R, 219Q	7.9	10I, 36I
11	Plasma	0	—	0	N
		1.5	67N, 70R, 219Q		
		24	67N, 69N/D, 219Q		
	PBMC	0	N	0	N
12	Plasma	0	184V, 62V	0	10I, 48V, 71T, 77I, 82A, 90M
		2	—	2.2	71T, 77I
	PBMC	0	184V, 62V	0	10I, 48V, 71T, 77I, 82A, 90M
		2.2	—	5	71T, 77I
13	Plasma	0	67N, 184V	0	10I, 46I, 71V, 77I, 88S
		1	67N, 184V	1	10I, 36M/I, 71V/T
		3.5	—	2.3	10I, 71V/T
	PBMC	0	67N/D, 184V/M	0	10I, 46M/I, 71V/T, 77I
		13.8	—	7.4	10I, 36I/M, 71V/A
14	Plasma	0	184V	0	10I, 20R, 24I, 36I, 53L, 54V, 71T, 82A
		1	—	1	—
	PBMC	0	N	0	10I/L, 20R/K, 24I/L, 36I, 53L/F, 54V, 71V/A, 82V/A
				6.2	—
15	Plasma	0	67N, 70R, 219Q	0	N
		1	67N, 70R		
		20.7	—		
	PBMC	0	67N, 70R, 219Q	0	N
		22.5	67N, 219Q		
16	Plasma	0	67N/D/G, 69A/D, 70R, 219Q	0	N
		19.5	219Q		
	PBMC	0	69A/D, 70R, 219Q	0	N
		19.5	69A/D, 219Q		

[a]—, wild type.
[b]This patient died at this time point with RT mutations detected.
[c]N, no resistance mutations.
[d]New ART was introduced at the time.

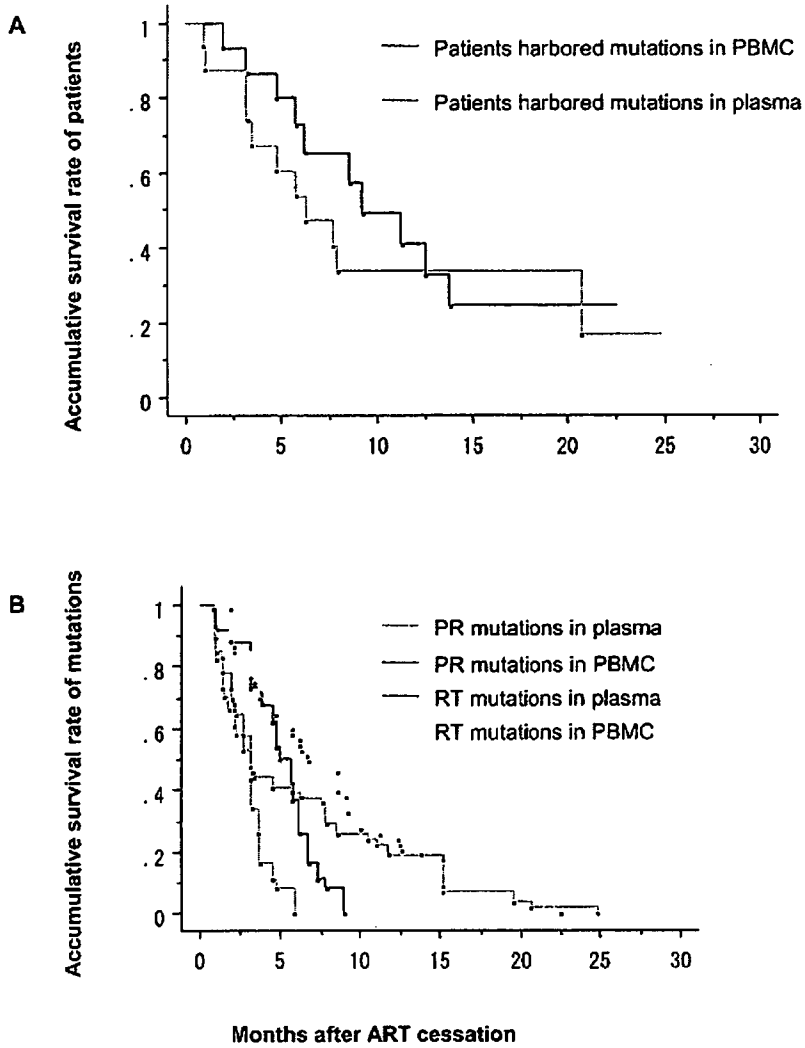

FIG. 1. (A) Kaplan–Meier curves showing percent of 16 patients with drug resistance mutations in plasma or PBMC. (B) Kaplan–Meier curves showing percent of 133 drug resistance mutations (59 RT and 74 PR) in plasma or PBMC. PR, in plasma vs. in PBMC ($p < 0.05$); RT, in plasma vs. in PBMC ($p < 0.05$); PR vs. RT ($p < 0.05$).

We designed the present study with the main objectives of determining the duration of the reversal period from the presence of resistant viruses to wild-type viruses and of elucidating the reversal patterns of plasma- and PBMC-derived viruses after discontinuation of ART. To determine the duration of the reversal period (i.e., from resistant mutations of RT and PR genes of plasma viruses and proviruses to wild type), sequential specimens of plasma and PBMC from patients with resistance mutations were sequenced after ART was interrupted. We found that the PR and RT resistance mutations shifted to wild type much more rapidly in plasma than in PBMC after ART cessation. In 3.2 months after ART stopped, 50% of the resistance mutations in plasma-derived viruses shifted to wild type and 50% of the major mutations of both RT and PR regions were undetected by direct sequencing. This period was similar to that reported by other investigators.[13,14,16–20] However, 50% of the mutations of RT and PR were detected by 6.7 and 5.7 months, respectively, when PBMC samples were used. Accordingly, when the patient develops virologic failure and drug resistance testing is performed using plasma sample after 3.2 months of ART cessation, the results of the test should be interpreted with caution, especially when deciding subsequent ART regimens, because 50% of mutation residues were undetectable by testing. When a resistant virus is not detected by drug-resistant testing, therapy using the same antiretroviral drugs or the same class of agents that reveal cross resistance is usually associated with early drug failure by previously acquired resistant viruses.[23,24] Therefore, like other recommendations,[1,2] drug-resistance testing should be performed soon after ART cessation. However, according to our data, the testing period could be postponed for 2.5 months (from 3.2 to 5.7 months) after ART withdrawal if PBMCs are used instead for plasma. In this regard, PBMC is a suitable candidate specimen for drug-resistance testing during off therapy.

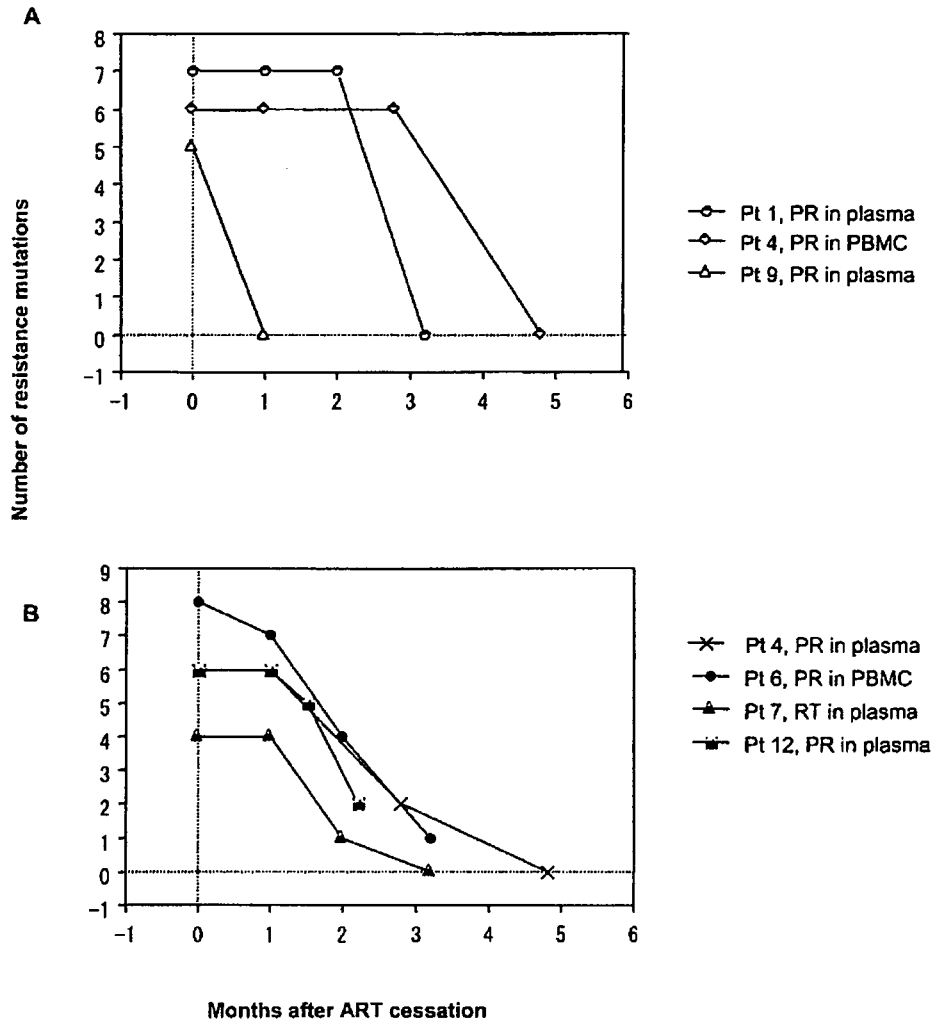

FIG. 2. Two Representative patterns of resistance mutations reverted to wild type after ART cessation. (A) Steep disappearance pattern of resistance mutations; (B) gradual reversal pattern of resistance mutations. RT, reverse transcriptase; PR, protease.

Drug-resistance testing is not advised for patients with VL <1000 copies/ml since amplification of the virus is unreliable.[1,2] However, if ART has to be discontinued because of ART-related toxicities and VL was undetectable at the time of discontinuation, the timing of the test is a practical question. Others report[17] a sharp reduction in the number of mutations at the time of viral load increase in patients during structured treatment interruption. Our results showed that at 1 month after ART cessation, VL dramatically increased from <1000 copies/ml to >4 $\log_{10}$ copies/ml in 6 patients who stopped treatment due to causes other than ART failure. However, the rebounded viruses in 5 of these 6 patients were still resistance mutant but not the wild-type virus. We previously reported that drug resistance mutations emerged gradually when therapy failed.[10] In contrast, the results here showed that nearly 50% of the mutations disappeared abruptly when ART completely stopped. Thus, waiting for several months after ART withdrawal until stabilization of the VL may potentially result in missing important information for selecting the subsequent therapeutic regimen. Therefore, in such situations, drug-resistance testing should be performed after 1 month to obtain a reliable result after ART withdrawal.

We previously studied the emergence of drug resistance during therapy and reported that the appearance of drug resistance in plasma viruses precedes that in proviruses by more than 1 year and recommended the use of plasma samples for drug-resistance testing during therapy.[10] Considering the high concordance of resistance mutations between plasma and PBMC, and the long persistence period of mutations in PBMC, we conclude that when ART stopped, if PBMC could be used as the sample for resistance assay, the test period may be postponed for 3 months.

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Novel Mutation of Human DNA Polymerase γ Associated with Mitochondrial Toxicity Induced by Anti-HIV Treatment

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(See the editorial commentary by Lewis, on pages 1399–401.)

Mitochondrial toxicity is a major adverse effect of the nucleoside reverse-transcriptase inhibitors (NRTIs) used for treatment of human immunodeficiency virus type 1 (HIV-1) infection and can result in life-threatening lactic acidosis. The toxicity is due to inhibition of polymerase γ (Pol γ), which is required for replication of mitochondrial DNA (mtDNA). Genetic factors could be involved in this process, given that not all NRTI-treated patients experience the toxicity. In 1 patient with lactic acidosis, a novel homozygous Pol γ mutation (arginine to cysteine at codon 964 [R964C]) was identified at a site close to polymerase motif B, which is highly conserved among family A polymerases. Recombinant R964C Pol γ showed only 14% activity, compared with that of wild-type Pol γ. Culture with stavudine significantly reduced mtDNA levels in patient-derived lymphoblastoid cell lines (LCLs) harboring R964C Pol γ, compared with those in LCLs harboring wild-type Pol γ. The novel Pol γ mutation could be associated with the severe lactic acidosis induced by long-term NRTI use.

Today's antiretroviral regimens are highly effective at suppressing HIV-1 replication and restoring immune function. However, long-term use of some antiretroviral agents is often associated with a variety of toxicities that can decrease quality of life or jeopardize the patient's health [1, 2]. The nucleoside reverse-transcriptase inhibitors (NRTIs) that represent the backbone of current anti–HIV-1 regimens are associated with a variety of long-term adverse effects, most of which are attributed to mitochondrial toxicity, possibly due to inhibition of mitochondrial DNA (mtDNA) replication. mtDNA replicates by a multienzyme complex, the main component of which is the nuclear-encoded DNA polymerase γ (Pol γ) [3]. NRTIs are thought to induce mitochondrial toxicity by inhibiting Pol γ, which results in the depletion of mtDNA, damage of the respiratory chain, elevation of serum lactate levels, and life-threatening lactic acidosis [3–6].

The antiretroviral agent stavudine (d4T) was once used but later dropped from the preferred first-line combination regimens because of its high mitochondrial toxicity [1, 2]. In vitro studies showed that d4T causes the greatest inhibition of Pol γ activity, and clinical studies showed that d4T use is most significantly associated with the elevation of serum lactate levels among clinically used NRTIs [4, 5, 7, 8]. However, d4T is a component of GPO-VIR and Triomune, which are widely used generic drugs in resource-limited situations, and it is still commonly prescribed, especially in developing countries [9, 10]. Furthermore, not only

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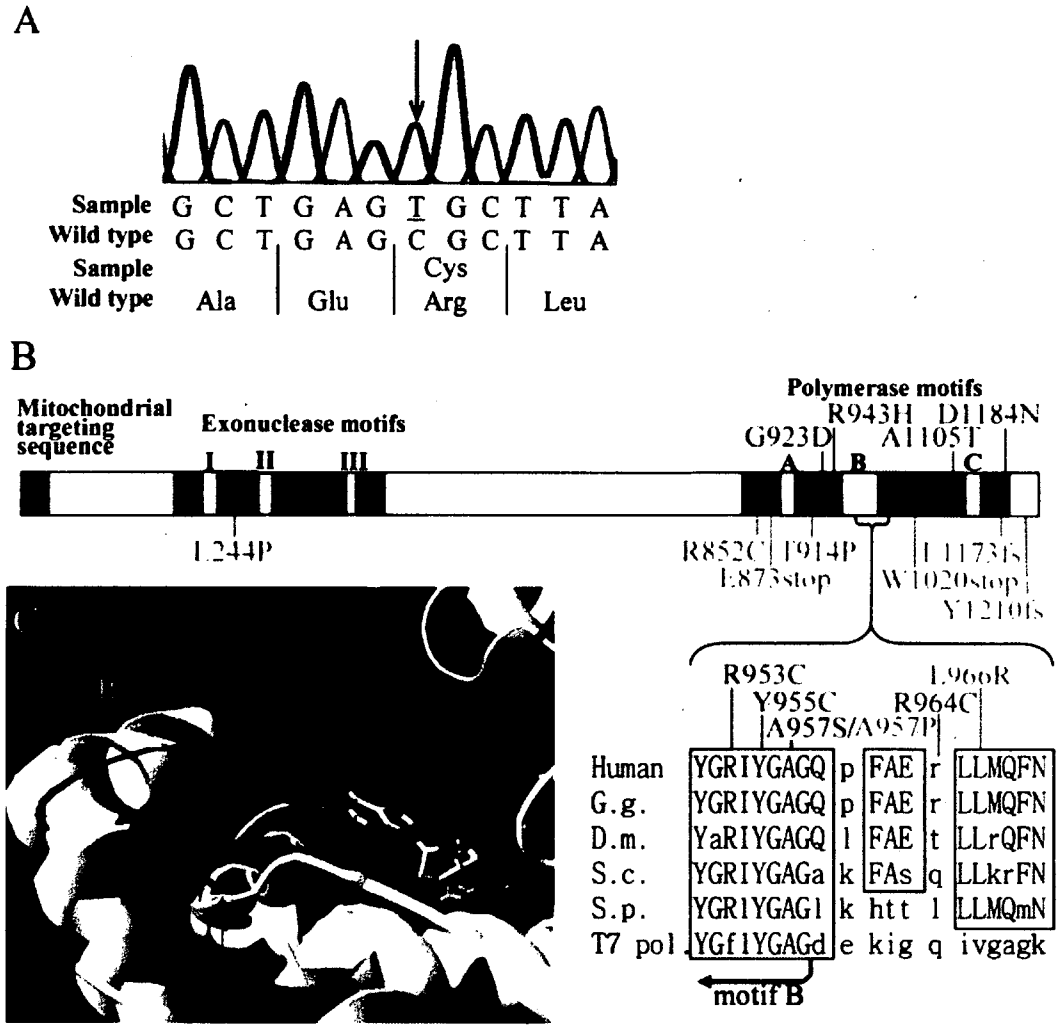

Figure 1. A novel polymerase γ (Pol γ) mutation, R964C, close to polymerase motif B. *A*, Direct sense-strand sequence around the R964C mutation in Pol γ. Ala, alanine; Arg, arginine; Cys, cysteine; Glu, glutamic acid; Leu, leucine. *B*, Novel and reported mutations in Pol γ. Active sites of exonuclease and polymerase are shown in green boxes. Mutations shown in green are associated with Alpers syndrome, and mutations shown in blue are associated with autosomal dominant progressive external ophthalmoplegia (PEO). G.g., *Gallus gallus*; D.m., *Drosophilia melanogaster*; S.c., *Saccharomyces cerevisiae*; S.p., *Schizosaccharomyces pombe*. *C*, Homologous-structure modeling of the Pol γ active site from the T7 polymerase complex structure with incoming ddATP. Motif B, O helix, is shown in pink, and motif A is shown in blue. The position of autosomal dominant PEO and Alpers syndrome mutations are shown in green. Position 964 is shown in yellow. Primer and template DNA strands are shown in red and green, respectively.

d4T but didanosine and zidovudine (AZT), both of which are often used in salvage therapy after virological treatment failure, also cause significant mitochondrial toxicity [4, 5, 7]. Therefore, mitochondrial toxicity is still a major critical problem in the management of patients treated with antiretroviral regimens [1, 2].

Because not all patients receiving long-term NRTI treatment experience mitochondrial toxicity, genetic factors as well as other environmental conditions could be involved. Human DNA Pol γ is composed of a 140-kDa catalytic subunit and a 55-kDa accessory subunit. Mutations in the gene for the catalytic subunit *(POLG)* have been shown to be a frequent cause of mitochondrial disorders, including progressive external ophthalmoplegia (PEO), which is often associated with multisystemic disorders (such as deafness, cataracts, depression, dysphagia, hypogonadism, neuropathy, and sensory ataxia) and Alpers syndrome (a fatal childhood disease caused by brain and liver failure often associated with refractory seizures, episodic psychomotor regression, cortical blindness, and liver disease with micronodular cirrhosis) [11–15]. The main hypothesis of the present study was that genetic variations in *POLG* promote sensitivity to NRTI treatment. To test our hypothesis, we se-