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Development of HIV with Drug Resistance after CD4 Cell Count–Guided Structured Treatment Interruptions in Patients Treated with Highly Active Antiretroviral Therapy after Dual–Nucleoside Analogue Treatment

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(See the editorial commentary by Arduino on pages 735–7)

Background. For patients with human immunodeficiency virus (HIV) infection, structured treatment interruption (STI) is an attractive alternative strategy to continuous treatment, particularly in resource-restrained settings, because it reduces both side effects and costs. One major concern, however, is the development of resistance to antiretroviral drugs that can occur during multiple cycles of starting and stopping therapy.

Methods. HIV genotypic drug resistance was investigated in 20 HIV-infected Thai patients treated with highly active antiretroviral therapy (HAART) and CD4 cell count–guided STI after dual nucleoside reverse-transcriptase inhibitor (NRTI) treatment. Resistance was tested at the time of the switch from dual-NRTI treatment to HAART and when HAART was stopped during the last interruption.

Results. After STI, one major drug-resistance mutation occurred (T215Y), and, in the 4 samples with preexisting major mutations (D67N [$n = 2$], K70R [$n = 2$], T215Y [$n = 2$], and T215I [$n = 1$]), the mutations disappeared. All mutations in the HIV protease gene were minor mutations already present, in most cases, before STI was started, and their frequency was not increased through STI, whereas the frequency of reverse-transcriptase gene mutations significantly decreased after the interruptions. After the 48-week study period, no patients had virological failure. Long-term follow-up (108 weeks) showed 1 case of virological failure in the STI arm and 1 in the continuous arm. No virological failure was seen in patients with major mutations.

Conclusions. Major HIV drug-resistance mutations were not induced through CD4 cell count–guided treatment interruptions in HIV-infected patients successfully treated with HAART after dual-NRTI therapy.

The introduction of HAART has dramatically improved the course of HIV infections [1, 2]. However, with HAART being widely used, long-term toxicity occurs [3, 4]. Although the therapy is cost-effective in some developed countries [5, 6], the costs of antiretroviral drugs impose a major economic burden on countries with limited resources like Thailand [7]. Structured treatment interruption (STI) is, therefore, an attractive alternative to continuous treatment, reducing both side effects and costs. One major concern, however, is the emergence of HIV with resistance to antiretroviral drugs that can occur during multiple cycles of starting and stopping therapy. Previous clinical trials investigating STI in the treatment of well-controlled chronic HIV infection did not demonstrate an increased occurrence of resistance [8–12]. However, new data demonstrate the appearance of resistance during STI. A trial

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with long-cycle STI, which is more similar to CD4 cell count–guided treatment interruptions, and the use of efavirenz-based HAART was stopped because of the occurrence of resistance in 3 of 8 patients [13]. In another similar study with various HAART regimens, resistance developed in 21.3% of patients who receive STI [14]. In a trial with repeated cycles of STI, M184V and L90M mutations could be detected in minor virus populations at different times during the trial [15]. Furthermore, the week-on, week-off arm of a large treatment-interruption trial had to be discontinued because of virological failure [16]. Thus, the risk of emergence of resistance might have been underestimated so far. In addition, these trials were performed with patients who had no history of previous failure to respond to antiretroviral therapy or in a mixed population, restricting the population of patients who might profit from STI. Previous results had shown that the week-on, week-off strategy had to be stopped because of virological failure also in patients previously treated with a dual nucleoside reverse-transcriptase inhibitor (NRTI) regimen [17]. We therefore investigated genotyping resistance in patients previously treated with dual-NRTI therapy who were being successfully treated with HAART and were undergoing CD4 cell count–guided STI, probably the most-promising interruption strategy for such patients.

PATIENTS AND METHODS

STI for patients previously treated with dual-NRTI therapy followed by HAART was investigated by the HIV Netherlands, Australia, Thailand Research Collaboration (HIV-NAT) of the Thai Red Cross AIDS Research Centre. An open-label, randomized 3-arm study was conducted to evaluate and compare the efficacy, safety, and tolerability of the following regimens: (1) continuous treatment with saquinavir–soft gel capsule, 1600 mg once per day, plus ritonavir, 100 mg once a day, plus 2 NRTIs (either zidovudine and lamivudine or stavudine and didanosine; (2) STI with the same regimen that was suspended and restarted on alternating weeks of week on, week off; and (3) STI with the same regimen that was suspended and restarted on the basis of CD4 cell count–driven criteria (from study HIV-NAT 001.4 [17]), in which therapy was stopped if the CD4 cell count decreased to <350 cells/μL or to 30% of preinterruption level, depending on the patient's choice. Patients qualified for the study if their last CD4 cell count was >350 cells/μL and their viral load had been <50 copies/mL for at least 6 months prior to enrollment. The "week-on, week-off" arm had to be discontinued because of virological failures at week 72 [17]. This strategy was not investigated further.

Ethics. The study was performed in accordance with the approval of the ethics committee of Chulalongkorn University, which conforms to the Helsinki Declaration of 1975, as revised in 1983.

Participants. The participants were HIV-infected Thai patients who had participated in the HIV-NAT 001 trial series that started in 1997, when they were treated with dual-NRTI therapy that consisted of zidovudine and zalcitabine at either the standard or half the standard dosage for 48 weeks, as described in a previous publication [18]. Initial treatment was followed by 3 years of saquinavir-based HAART [19]. If the viral load remained undetectable (<50 copies/mL) and the CD4 cell count was >350 cells/μL, a patient was enrolled in the study after written informed consent had been obtained.

Procedures. During the 48-week observation period, patients randomized to either of the STI arms had an evaluation every 2 months, and frozen plasma samples were obtained for further analysis. To assess whether resistance was acquired during multiple cycles of CD4 cell count–guided treatment interruptions, HIV genotyping resistance testing was performed on the first plasma sample with a viral load of >1000 copies/mL that was obtained after treatment was stopped during the last cycle of treatment interruption, for the CD4-guided arm. The "week-on, week-off" arm was not investigated further, as this strategy showed too many virological failures. To differentiate preexisting resistance from acquired resistance, viral genotypic resistance testing was done on the last plasma sample obtained before patients were switched from dual-NRTI therapy to protease inhibitor–based HAART. The methodology for HIV reverse-transcriptase genotyping has been described elsewhere [20]. For the protease genotypic resistance assay, the same methodology was used but with the following primers: PI-1685 (5′-GGAATTTTCCTCAGAGCAG-ACCAG-3′), PI-2209 (5′-TCTTCTGTCAATGGCCACTGTTT-AAC-3′), and PI-2172 (5′-CCATTCCTGGCTTTAATGTTACT-GGTAC-3′). We categorized major and minor mutations in accordance with guidelines set by the International AIDS Society–US Drug Resistance Mutations Group [21].

Poststudy follow-up during STI totaled 108 weeks. For the final 12 weeks, all patients were treated with continuous HAART. Virological failure was defined as a viral load of >500 copies/mL at any time, for the continuous-treatment arm, and after 3 months of continuous HAART, for the STI arm.

Statistical analysis. Descriptive results are presented as means $\pm$ SD, medians with interquartile range (IQR), and percentages. Inferential statistics using either parametric or nonparametric tests were used, as appropriate for the data type. The overall level of significance was set at $\alpha = 0.05$. To compare means, medians, and proportions of investigated factors between groups with and groups without resistance, the Student's t test, Mann-Whitney U test, and χ^2 test were used. Fisher's exact test was used for small values for which the χ^2 test was not applicable. Statistics were done using the SPSS software, version 9.0 (SPSS).

RESULTS

Results for the primary end points of this study have been presented elsewhere [17]. In summary, 74 NRTI-pretreated patients were included in the study, and continuous HAART was compared with CD4 cell count–guided STI. "Week-on, week-off" STI treatment was discontinued ahead of schedule, because failures were unacceptably frequent (occuring in 12 [46%] of 26 patients) [17, 22]. At the time of virological failure, no HIV with drug resistance was found in plasma samples from 3 patients, samples from 2 patients were not amplifiable, and, for 1 patient, no testing was done. Mutations in the HIV reverse-transcriptase gene were found in samples from 3 patients (at position 215 [$n = 3$] and positions 219, 210, 67, and 41 [$n = 1$ for each]). Mutations in the HIV protease gene were detected in samples from 4 patients (at position 63 [$n = 3$] and 82 [$n = 1$]). Of note, all patients had a viral load of <50 copies/mL after reinitiating continuous HAART. The "week-on, week-off" strategy was unreliable and thus not investigated further. Twenty-three patients were treated with CD4-guided STI, and 25 patients were treated with continuous HAART. The observational period was 48 weeks, and follow-up continued for 108 weeks. After 108 weeks (96 weeks of STI and 12 weeks of continuous treatment), 1 case of virological failure, defined as a viral load >500 copies/mL, occured after 3 months of continuous treatment, in a patient with nonadherence in the STI group [22]. Another failure occurred in the continuous treatment group at week 96; no results of resistance testing were available for this patient.

Frozen plasma samples obtained before HAART were available from 20 (87%) of the patients, and samples obtained after STI were available from 20 (87%) of the patients. Baseline characteristics of these patients are shown in table 1. Of note, only 30% of patients had a VL < 400 copies/mL before switching from dual-NRTI (zidovudine/zalcitabine) treatment to HAART. The NRTI regimen was changed from zidovudine/zalcitabine to zidovudine/lamivudine for 9 patients and to stavudine/didanosine for 11 patients. The female-to-male sex ratio was balanced, and the mean age (±SD) was 35.4 ±6.4 years. Most patients (55%) were asymptomatic before starting antiretroviral therapy. Depending on their reaction to STI, the patients underwent 1–3 (mean, 1.65) treatment interruptions during the 48-week observation period. Resistance testing was performed at a median of 32 days (IQR, 28–63.5 days) after treatment was stopped during the last treatment interruption within the 48-week observation period.

Table 2 shows the results of HIV genotyping before HAART and after CD4 cell count–guided STI. Genotyping could be done on 11 samples obtained before HAART, and 9 samples could not be amplified. Three of these 9 samples had a viral load of <400 copies/mL, and, in 6 other samples, RNA degradation had occurred. In 11 samples obtained before the switch from dual-NRTI treatement to HAART, mutations were found in 4 (36%), and no mutation was present in 7 (64%). In 17 samples obtained after CD4 cell count–guided STI, resistance mutations were found 9 (53%). Three of these 17 samples could not be amplified, possibly because of mutations at the primer site. HIV mutations present before the start of protease inhibitor–based HAART and detected after STI are shown in figure 1 and table 2. Major mutations were found in 4 (36%) of 11 samples obtained before HAART and in 1 (6%) of 17 samples obtained after STI ($P = .062$, by Fisher's exact test). The matched pair comparison showed that minor mutations in the HIV protease gene that were present before HAART were also found after STI. Minor mutations emerged in HIV from 4 samples, and, in HIV from 1 sample, 1 mutation was lost. New mutations were acquired by HIV in 2 samples, and 2 samples obtained before HAART could not be amplified. Preexisting mutations in the HIV reverse-transcriptase gene disappeared after STI. In 2 patients with this HIV mutation pattern, the virus reverted to wild type, whereas, in 2 others, secondary mutations which do not lead to drug resistance were present after the treatment interruption. Only 1 major mutation (T215Y) occurred after STI, in a patient with HIV that did not have genotypic resistance before HAART. As shown in figure 2, the frequency of mutations in the HIV reverse-transcriptase

Table 1. Baseline characteristics of the 20 patients enrolled in the CD4 cell count–guided structured treatment interruption (STI) arm from whom plasma samples were available.

Characteristic	Value
Demographic	
Age, mean years ±SD	35.4 ±6.4
Sex	
Females	9 (45)
Males	11 (55)
CDC HIV disease stage	
CDC A	11 (55)
CDC B	8 (40)
CDC C	1 (5)
CD4 cell count, cells/μL (IQR)	
Before ART	380 (150–563)
Before HAART	732 (550–872)
Before STI	766 (550–872)
Viral load	
Median level before ART, $\log^{10}$ copies/mL (IQR)	4.8 (4.1–5.4)
Level <400 $\log^{10}$ copies/mL before HAART	7 (30.4)
HAART	
No. of STI cycles, mean ±SD	1.65 ± 0.67
NRTIs included	
Zidovudine/lamivudine backbone	9 (45)
Stavudine/didanosine backbone	11 (55)

NOTE. Data are no. of patients (%), unless otherwise indicated. ART, antiretroviral treatment; CDC, Centers for Disease Control and Protection; NRTI, nucleoside reverse-transcriptase inhibitor.

Table 2. Drug-resistance profile of HIV in patients who received structered treatment interruption (STI), as determined before HAART and after the last cycle of STI.

	Before HAART			After last cycle of STI			
NRTIs included in HAART	CD4 cell count, cells/μL	Viral load, copies/mL	Mutation(s)[a]	CD4 cell count, cells/μL	Viral load, copies/mL	Mutation(s)[a]	No. of STI cycles
D4T/DDI	113	<400	NA	554	569	None	2
AZT/3TC	835	2083	None	348	212,000	**T215Y**	1
D4T/DDI	872	2829	NA	872	59,600	NA	1
AZT/3TC	426	1591	NA	530	2250	NA	1
D4T/DDI	530	2339	None	384	18,100	None	2
AZT/3TC	581	488	None	533	40,400	*V77I*	2
AZT/3TC	419	<400	NA	360	61,400	NA	1
AZT/3TC	851	15,714	NA	746	80,700	*K20R*	2
D4T/DDI	956	1538	None	1183	19,800	None	2
D4T/DDI	1028	1077	NA	672	7070	None	2
D4T/DDI	599	1949	*L10I*	642	16,200	*L10I, L63P*	2
D4T/DDI	766	8378	NA	630	19,300	None	2
AZT/3TC	550	11,175	**D67N**, **K70R**, **T215Y**	351	9510	None	2
AZT/3TC	698	<400	NA	576	2870	*L63P*	3
D4T/DDI	1316	2216	**T215Y**, *L63P*	607	4320	*L63P*	3
AZT/3TC	814	2024	*L10V, K20R, L63P*	814	57,700	*K20R, L63P*	1
D4T/DDI	804	1309	**T215F**	804	214,000	None	1
D4T/DDI	664	<400	*L63P*	664	27,400	*L63P*	1
AZT/3TC	608	31,796	**D67N**, **K70R**, **T215I**, *L10V, L63S*	783	69,500	**K103T**, *L10V, L63S*	1
D4T/DDI	1082	1747	NA	1084	23,200	None	1

NOTE. AZT, zidovudine; DDI, didanosine; D4T, stavudine; NA, HIV not able to be amplified; 3TC, lamivudine.

[a] Mutations in bold are in the reverse-transcriptase gene, and mutations in italics are in the protease gene.

gene was significantly reduced, from 72% before HAART to 12% after STI ($P < .01$, by Fisher's exact test). The frequency of mutations in the protease gene did not significantly change (72% vs. 65% after STI).

During the 48-week observation period, the same HAART regimen remained effective in suppressing the viral load in all patients who had to restart therapy for at least 3 months, in accordance with the protocol criteria. Eighteen patients who reinitiated HAART achieved a viral load of <500 copies/mL after 3 months of continuous therapy. The median viral load rebound after STI was 4.6 $\log_{10}$ copies/mL (IQR, 3.9–4.9) in patients with drug-resistant HIV and 4.3 $\log_{10}$ copies/mL (IQR, 3.9–4.3) in patients without drug-resistant HIV. After posttrial follow-up of 108 weeks, 1 patient had a viral load of >500 copies/mL 3 months after reinitiating HAART, which qualified as virological failure. This failure occurred at week 72 because the patient did not adhere to reinitiated HAART. The patient demonstrated no major HIV mutations after STI and was discontinued from the study. At week 108, all patients had received 3 months of continuous HAART. All but the patient mentioned above had a viral load of <400 copies/mL [22]. The factors believed to predict the occurrence of HIV drug-resistance mutations after STI—presence of resistance before HAART, viral load rebound, CD4 cell count at the time of initiation of STI, number of STI cycles, CD4 cell count before antiretroviral therapy, CD4 cell count before HAART, viral load before HAART, sex, and the NRTIs included in the HAART—were investigated without significant results.

DISCUSSION

CD4 cell count–guided STI was tested in 23 HIV-infected Thai patients who had previously been treated with dual-NRTI therapy. Samples from 20 patients were available for HIV genotypic resistance testing. The patients were switched to HAART for 3 years and had a viral load of <50 copies/mL before enrollment. Before HAART, antiretroviral–associated major HIV reverse-transcriptase mutations were found in 4 (36%) of 11 samples, and minor mutations were found in 5 (45%) of 11 samples. After CD4 cell count–guided treatment, only 1 (6%) of 17 had

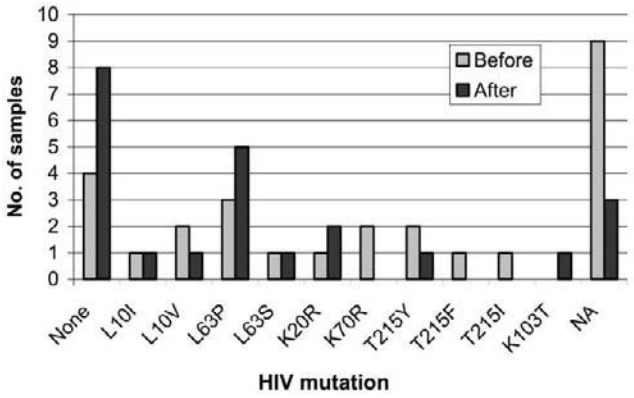

Figure 1. HIV drug-resistance mutations detected in plasma samples obtained from patients before HAART (before) and after structured treatment interruption (after). NA, not able to be amplified.

major mutations, and minor mutations were found in 8 (47%) of 17 samples. No virological failure was seen in patients with major HIV mutations after 108 weeks of follow-up.

HIV with drug resistance is a major concern in STI and has been shown to occur in clinical trials investigating structured interruptions of HAART [13, 15, 16, 23]. On the other hand, it also has been shown that interruption of HAART can convert a resistant virus population to wild-type virus [24–27]. Although this strategy has been harmful in the patients receiving salvage therapy [28], the situation may be different in immunologically intact patients infected with HIV resistant to NRTIs but treated successfully with HAART. Indeed, major HIV mutations present after dual-NRTI therapy and before HAART had disappeared after STI. Seventy percent of our patients had a detectable viral load of > 400 copies/mL after dual-NRTI therapy. Despite having undetectable viral load after HAART, they can be considered a population at risk for resistance development after STI. However, a major HIV mutation was found in only 1 patient after STI, and this did not lead to virological failure during a follow-up period of 108 weeks. To our knowledge, this study is the first investigation of STI done exclusively in HIV-infected patients with viral supression who are treated with HAART after dual-NRTI therapy.

In this CD4 cell count–guided treatment-interruption trial, HIV mutations were frequently observed after STI in patients previously treated with dual-NRTI therapy before HAART. However, only 1 of these mutations (T215Y) was a major mutation. HIV from 8 patients displayed 1 or 2 secondary mutations, which alone do not result in resistance to antiretrovirals. They probably represent naturally occurring polymorphisms rather than drug-selected mutations. Indeed, the minor HIV protease gene mutations seen after STI were nearly all present before initiation of HAART. Major mutations seen after STI involved the reverse-transcriptase gene, whereas minor mutations involved polymorphic positions coding for the protease gene. In our study, positions 10, 20, 63, and 77 were involved. Although these mutations do not cause significant drug resistance by themselves, some of them contribute to drug resistance when present together with other protease mutations [29, 30]. Whether further HIV mutations will be acquired and thus lead to clinical drug resistance after additional cycles of STI cannot be determined from the data available at present. But none of the patients, except for the one who did not adhere to HAART, developed virological failure by week 108 of follow-up. One other case of virological failure occurred in the continuous treatment arm [22]. Deeks et al. [31] have shown that durable suppression of the HIV virus population may be achieved with a combination regimen containing only 1 fully active agent. Patients infected with HIV that developed major mutations still had at least 1, and usually 2, drugs that were still active against the strain, which could explain why even patients with resistant HIV responded well to reinitiation of HAART after treatment interruption. How sustainable viral suppression will be remains to be seen. Nevertheless, after 108 weeks of follow-up and with only 1 treatment failure, CD4 cell count-guided STI appears to be safe in this patient population. Accordingly, all patients with major HIV mutations in the "week-on, week-off" arm whose treatment failed had a viral load of <50 copies/mL after reinitiation of HAART. Using a univariate model, we found no factors predicting the development of HIV mutations. In accordance with a study performed in a very similar group of patients, multidrug resistance against NRTIs was frequently seen in patients predominantly infected with HIV subtype A/E for whom NRTI treatment failed [20]. In our study, presence of preexisting mutations was not associated with development of resistance after STI. However, when mutations were present before initiation of HAART, we observed either reversion to wild-type virus or substitution of these mutations with minor mutations during STI. Interestingly, the frequency of reverse-transcriptase gene mutations significantly decreased after STI,

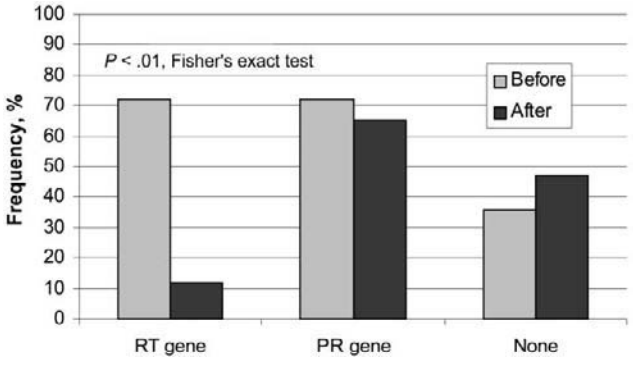

Figure 2. Frequency of mutations in the reverse-transcriptase (RT) and protease (PR) genes, defined as the total number of mutations divided by the number of amplified plasma samples, before HAART (before) and after structured treatment interruption (after).

and there was a trend toward a reduction in the number of preexisting major mutations in the HIV reverse-transcriptase gene after STI. Whether these mutations truly disappeared or whether we just measured overgrowth of wild-type virus is difficult to determine. The time from the stopping HAART to the performance of genotyping was 32 days, and a previous study has shown that, within the first 30 days, most reverse-transcriptase mutations remain [25]. Although we cannot conclude from our data that CD4 cell count–guided STI is beneficial for such patients, we did not find any harmful effects of STI on the virological outcome.

The study has some limitations. The strength of the results and conclusions is somewhat diminished by the limited sample size. Because of RNA degradation, 6 samples could not be amplified. Another 3 samples could not be amplified due to probable mutations at the primer site. During the last treatment-interruption cycle, half of our patients had genotyping performed >30 days after stopping HAART (median, 32 days). It is possible that mutated viruses were not detected because of the relative increase in the number of wild-type viruses. However, Miller et al. [25] showed that mutations in the reverse-transcriptase gene remain largely unchanged 30 days after treatment interruption. Moreover, all major HIV mutations in our study population were mutations in the reverse-transcriptase gene. The observed decrease in major mutations may also have been due to random effects caused by small sample size and nonamplification of samples, rather than STI.

Nevertheless, the study offers an interesting insight into the effect of STI on patients who have received suboptimal antiretroviral therapy before HAART, and it is the first investigation that exclusively studied patients previously treated with dual-NRTI therapy. The long clinical follow-up of 108 weeks strengthens many of the conclusions and also supports the further use of CD4 cell count–guided STI in controlled conditions for patients with preexisting major HIV mutations. This is relevant, since STI may become an important treatment strategy in resource-limited places like Thailand, where many patients have previously been treated with dual-NRTI therapy and thus harbor HIV with major mutations in the reverse-transcriptase gene [20].

In conclusion, major HIV mutations were not induced through CD4 cell count–guided treatment interruptions in HIV-infected patients successfully treated with HAART after dual-NRTI therapy. However, the power of this trial is limited, and a cautious follow-up is necessary. The prevalence of infection with HIV with major mutations conferring drug resistance by themselves decreased from 36% of patients before CD4 cell count–guided STI to 6% after STI. Also, the mutations did not correlate with virological response to HAART so far, and the presence of preexisting mutations after dual-NRTI therapy did not negatively influence the virological outcome of STI. These findings only apply for the chosen CD4 cell count–guided treatment interruption strategy and cannot be extrapolated to other interruption strategies.

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