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

Reto Nuesch, Jintanat Ananworanich, Sunee Sirivichayakul, Sasiwimol Ubolyam, Umaporn Siangphoe, Andrew Hill, David Cooper, Joep Lange, Praphan Phanuphak, and Kiat Ruxrungtham

HIV Netherlands, Australia, Thailand Research Collaboration (HIV-NAT), Thai Red Cross AIDS Research Centre, Bangkok, Thailand; Outpatient Department of Internal Medicine and Division of Infectious Diseases, University Hospital Basel, Basel, Switzerland; Department of Medicine, Chulalongkorn University, Bangkok, Thailand; University of Liverpool, Liverpool, United Kingdom; National Centre in HIV Epidemiology and Clinical Research, Sydney, Australia; and International Antiviral Therapy Evaluation Center, Amsterdam, The Netherlands

(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 pre-existing major mutations (D67N, K70R, T215Y, and T215I), 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
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-inter-
ruption trial had to be discontinued because of virological fail-
ure [16]. Thus, the risk of emergence of resistance might have
been underestimated so far. In addition, these trials were per-
formed with patients who had no history of previous failure
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 in-
vestigated 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 fol-
lowed 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, ran-
domized 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 pa-

tients 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 de-
scribed 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, pa-

tients 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 per-

formed on the first plasma sample with a viral load of >1000
copies/mL that was obtained after treatment was stopped dur-

ing the last cycle of treatment interruption, for the CD4-
guided arm. The “week-on, week-off” arm was not investi-
gated 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′-GGAAATTTTCTCAGAGCAG-
ACCAG-3′), PI-2209 (5′-TCTTCTGCTAATGGCCACTGGT-
AACC-3′), and PI-2172 (5′-CCATTCTGGCTTTAATGTCACT-
GGTAC-3′). We categorized major and minor mutations in
accordance with guidelines set by the International AIDS So-
ciety–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 ± SD, medians with interquartile range (IQR), and
percentages. Inferential statistics using either parametric or
non-
parametric tests were used, as appropriate for the data type.
The overall level of significance was set at α = 0.05. To compare
means, medians, and proportions of investigated factors be-
tween groups with and groups without resistance, the Student’s
t test, Mann-Whitney U test, and χ² test were used. Fisher’s
effect test was used for small values for which the χ² 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 (occurring 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 detected in samples from 4 patients (at position 63 [n = 1] for each). Mutations in the HIV protease gene were found in samples from 3 patients (at position 215 [n = 3] and positions 219, 210, 67, and 41 [n = 1 for each]). In 3 of 17 samples (18%), resistance to at least 1 NRTI, nucleoside reverse-transcriptase inhibitor. As shown in figure 1 and table 2. Major 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 HAART (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.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
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<tbody>
<tr>
<td><strong>Demographic</strong></td>
<td></td>
</tr>
<tr>
<td>Age, mean years ± SD</td>
<td>35.4 ± 6.4</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>9 (45)</td>
</tr>
<tr>
<td>Males</td>
<td>11 (55)</td>
</tr>
<tr>
<td><strong>CDC HIV disease stage</strong></td>
<td></td>
</tr>
<tr>
<td>CDC A</td>
<td>11 (55)</td>
</tr>
<tr>
<td>CDC B</td>
<td>8 (40)</td>
</tr>
<tr>
<td>CDC C</td>
<td>1 (5)</td>
</tr>
<tr>
<td><strong>CD4 cell count, cells/µL (IQR)</strong></td>
<td></td>
</tr>
<tr>
<td>Before ART</td>
<td>380 (150–563)</td>
</tr>
<tr>
<td>Before HAART</td>
<td>732 (550–872)</td>
</tr>
<tr>
<td>Before STI</td>
<td>766 (550–872)</td>
</tr>
<tr>
<td><strong>Viral load</strong></td>
<td></td>
</tr>
<tr>
<td>Median level before ART, log10 copies/mL (IQR)</td>
<td>4.8 (4.1–5.4)</td>
</tr>
<tr>
<td>Level &lt;400 log10 copies/mL before HAART</td>
<td>7 (30.4)</td>
</tr>
<tr>
<td><strong>No. of STI cycles, mean ± SD</strong></td>
<td>1.65 ± 0.67</td>
</tr>
<tr>
<td><strong>NRTIs included</strong></td>
<td></td>
</tr>
<tr>
<td>Zidovudine/lamivudine backbone</td>
<td>9 (45)</td>
</tr>
<tr>
<td>Stavudine/didanosine backbone</td>
<td>11 (55)</td>
</tr>
</tbody>
</table>

**NOTE.** Data are no. of patients (%), unless otherwise indicated. ART, antiretroviral treatment; CDC, Centers for Disease Control and Protection; NRTI, nucleoside reverse-transcriptase inhibitor.
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 postrtrial follow-up of 108 weeks, 1 patient had a viral load of $1500$ 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, sexual, 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 $<500$ 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

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

<table>
<thead>
<tr>
<th>NRTIs included in HAART</th>
<th>Before HAART</th>
<th>After last cycle of STI</th>
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<tbody>
<tr>
<td></td>
<td>CD4 cell count, cells/μL</td>
<td>Viral load, copies/mL</td>
</tr>
<tr>
<td>D4T/DDI</td>
<td>113</td>
<td>&lt;400</td>
</tr>
<tr>
<td>AZT/3TC</td>
<td>835</td>
<td>2083</td>
</tr>
<tr>
<td>D4T/DDI</td>
<td>872</td>
<td>2829</td>
</tr>
<tr>
<td>AZT/3TC</td>
<td>426</td>
<td>1591</td>
</tr>
<tr>
<td>D4T/DDI</td>
<td>530</td>
<td>2339</td>
</tr>
<tr>
<td>AZT/3TC</td>
<td>581</td>
<td>488</td>
</tr>
<tr>
<td>D4T/DDI</td>
<td>419</td>
<td>&lt;400</td>
</tr>
<tr>
<td>AZT/3TC</td>
<td>851</td>
<td>15,714</td>
</tr>
<tr>
<td>D4T/DDI</td>
<td>956</td>
<td>1538</td>
</tr>
<tr>
<td>AZT/3TC</td>
<td>426</td>
<td>1591</td>
</tr>
<tr>
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<td>426</td>
<td>1591</td>
</tr>
</tbody>
</table>

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

* Mutations in bold are in the reverse-transcriptase gene, and mutations in italics are in the protease gene.
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 suppression 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.
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.

Acknowledgments

We thank all the patients for their participation.

Financial support. This work was supported mainly by the HIV-NAT intramural research fund, Thai Red Cross AIDS Research Centre, and was partially supported by the Chulalongkorn Medical Research Center, Chulalongkorn University, and by Roche (Welwyn, United Kingdom). R.N. was supported by a grant from the Swiss National Science Foundation.

Potential conflicts of interest. J.A. has received travel grants and honoraria from Hoffmann-LaRoche. A.H. is a former employee of Hoffmann-LaRoche. D.C. has received research grants/funding, honoraria, or lecture sponsorships from or is a consultant or advisor to Abbott, Boehringer-Ingelheim, Bristol-Myers-Squibb, Chiron, Gilead, GlaxoSmithKline, Pfizer, Roche, and Merck, Sharpe & Dohme. J.L. has received consultancy fees and honoraria from GlaxoSmithKline, Boehringer-Ingelheim, Bristol-Myers-Squibb, Hoffmann-LaRoche, Schering-Plough, Bayer, Shire Pharmaceuticals, Agouron/Pfizer, Virco/Tibotec, and Merck, Sharp & Dohme. P.P. has received honoraria from Bristol-Myers-Squibb, as a scientific consultant, and research grants from Bristol-Myers-Squibb, Hoffmann-LaRoche, GlaxoSmithKline, and Merck, Sharp & Dohme. K.R. has received travel grants, research grants, consultancy fees, and/or honoraria from Hoffmann-LaRoche, Bristol-Myers-Squibb, Gilead, Abbott, and Merck, Sharp & Dohme. All other authors: no conflicts.

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