Short communication

Evolution of HIV-1 genotype in plasma RNA and peripheral blood mononuclear cells proviral DNA after interruption and resumption of antiretroviral therapy

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Background: Structured antiretroviral therapy interruption (TI) is discouraged because of poorer AIDS and non-AIDS-related outcomes, but is often inevitable in clinical practice. Certain strategies could reduce the emergence of resistance mutations related to TI.

Methods: A total of 106 HIV-1-infected patients on stable HAART with undetectable plasma viral load were randomized to therapy continuation (n = 50) or CD4+ T-cell-guided TI (n = 56). Staggered interruption involved stopping non-nucleoside reverse transcriptase inhibitors (NNRTIs) 7 days before the nucleoside backbone. Genotypic resistance testing (GRT) was performed on proviral DNA from peripheral blood mononuclear cells (PBMCs) at baseline and before each TI, and on plasma RNA after each TI.

Results: At baseline, GRT on PBMCs detected mutations in nine patients and only two major mutations were identified. GRT on plasma samples performed after TIs showed nucleoside reverse transcriptase inhibitors (NRTI), NNRTI and protease inhibitor major resistance associated mutations in 10/56, 3/46 and 1/8 patients receiving these drugs, respectively. Only in two patients had the same mutations been observed in GRT on plasma after TI. Three patients presented virological failure after resumption of therapy, all receiving NNRTIs. In one of them, resistance mutations detected at failure had been also observed previously in GRT on plasma after TI.

Conclusions: Staggered interruption of NNRTIs 7 days before the nucleoside backbone does not avoid resistance emergence completely, but does not necessarily lead to virological failure after treatment resumption. Plasma HIV-1 RNA genotype after the interruption and the patient’s treatment history seem to be more useful than baseline proviral DNA genotype to assess the risk of virological failure after restarting therapy.

Introduction

Structured interruption of antiretroviral therapy (ART) is not a recommended treatment strategy. An increased risk of all-cause mortality, AIDS and serious non-AIDS events compared with continuous therapy has been demonstrated in clinical trials and cohort studies [1,2]. Furthermore, treatment interruption has been associated with the emergence of resistance mutations, which can lead to virological failure and a loss of future
therapeutic options [3–6]. However, in daily clinical practice, some patients might require ART interruption because of clinical complications, surgical procedures and other, non-medical conditions. The non-nucleoside reverse transcriptase inhibitors (NNRTIs), efavirenz and nevirapine, and some nucleoside reverse transcriptase inhibitors (NRTIs), such as lamivudine, are the drugs most commonly associated with emergence of resistance following treatment interruption because of their long half-life or low genetic barrier to resistance [3–6]. Simultaneous interruption of all drugs in a regimen including an NNRTI could allow exposure of actively replicating virus to selective pressure from non-suppressive NNRTI monotherapy, increasing the risk of resistance emergence [4]. It has been suggested that development of resistance could be reduced if NNRTIs were withdrawn some days before NRTIs, or if NNRTIs were replaced by another drug some days before treatment interruption [3]. In this report, we analyse the evolution of the HIV-1 genotype after treatment interruptions and the virological response after resumption of the same regimen during a 96-week follow-up of patients included in a CD4+ T-cell-guided randomized treatment interruption trial, in which NNRTIs were discontinued 7 days before NRTIs [7].

Methods

Study design and population

The STOPAR study is a randomized, open-label multicentre clinical trial conducted in Spain between January 2004 and May 2008 (registration code ISRCTN75856952) [7]. A total of 106 HIV-1-infected adults (older than 18 years) with a CD4+ T-cell count >500 cells/μl for at least 3 months and undetectable plasma viral load (RNA HIV-1<50 copies/ml) for at least 6 months, receiving stable HAART with two NRTIs plus one NNRTI (85%) or one protease inhibitor (PI; 15%) for at least 6 months, were randomized to therapy continuation (TC; n=50) or to CD4+ T-cell-guided therapy interruption (TI; n=56). Only one previous virological failure with confirmed or suspected resistance mutations was allowed. Patients in the TI group restarted ART, with the same regimen during a 96-week follow-up of patients included in a CD4+ T-cell-guided randomized treatment interruption trial, in which NNRTIs were discontinued 7 days before NRTIs [7].

Spanish Regulatory Authorities, and all patients signed an informed consent to participate.

Assessments

Clinical assessment and laboratory tests (plasma HIV-1 viral load, CD4+ T-cell count, and haematological and biochemical parameters) were performed at baseline, week 4, week 8, week 12 and every 12 weeks thereafter, up to 144 weeks. In patients taking ART, treatment adherence was assessed, on each visit, using a simplified version of a validated adherence questionnaire [8].

Virological failure was defined as two repeated determinations of plasma viral load (HIV-1 RNA)>50 copies/ml after resumption of ART.

Laboratory procedures

Plasma HIV-1 RNA was determined using a branched DNA assay (Versant HIV-1 RNA 3.0; Bayer Corporation, Tarrytown, NY, USA) or a real-time PCR technique (Abbott RealTime HIV-1; Abbott Laboratories, Abbott Park, IL, USA). Genotypic resistance tests (GRTs) were performed retrospectively on stored blood and plasma samples collected during the follow-up. In all patients in the TI arm, GRTs were performed on plasma samples collected after each interruption when viral load was >400 copies RNA/ml, and in patients in the TC arm only when virological failure occurred. In addition, GRTs on proviral DNA of peripheral blood mononuclear cells (PBMCs) were performed using patients’ blood samples collected at baseline and before each new treatment. Proviral DNA was successfully sequenced in 44 of 56 patients. DNA was extracted using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). PCR amplification was performed using a modification of a previously described protocol that excludes the reverse transcription step [9]. GRT in cell DNA or plasma (EDTA) RNA was done using a commercial sequencing method (ViroSeq HIV-1 Genotyping System Kit 2.0; Abbott Laboratories) according to the manufacturer’s specifications. Drug resistance-associated mutations were defined according to the International AIDS Society – USA guidelines [10].

As GRTs were performed retrospectively, clinicians were unaware of the emergence of resistance mutations after TI when the same antiretroviral regimen was resumed.

Results

The median age of the patients was 39.5 years, 73.6% were men and the baseline CD4+ T-cell count was 845 cells/μl (range 523–1,817 cells/μl). There were no differences in the clinical characteristics or laboratory results at baseline between the TC and TI groups.
A total of 90 (84.9%) patients were receiving an NNRTI-based regimen: 46 efavirenz (25 in the TI group) and 44 nevirapine (23 in the TI group). The most commonly used PI was lopinavir/ritonavir (7/16 of PI-treated patients).

Genotype resistance testing of PBMC samples at baseline
In the subset of patients assigned to TI with GRT on PBMC samples at baseline, minor resistance-related mutations or polymorphisms were detected in 9/44 (20%) patients and major resistance mutations in only two patients – one of them being T215S, a transition mutation considered a marker of the presence of the major mutation T215Y (Table 1). These patients with mutations detected by GRT on PBMC at baseline achieved viral suppression after resumption of the previous HAART.

Genotype resistance testing on plasma samples after TI
In all patients in the TI group, GRT was performed on plasma samples after each treatment interruption. In 10 of 56 (18%) patients, at least one major NRTI resistance mutation was detected by GRT on plasma after TI. Among patients with repeated interruptions, no new mutations different from those detected in the first GRT appeared. In seven patients (patients 101, 121, 204, 209, 305, 516 and 1,104), mutations emerging after TI were related to the interrupted drugs, and the M184V mutation, related to lamivudine resistance, was present in three patients. In the other patients, mutations were related to previous therapies, suggesting that they were archived mutations and not newly emerged ones (patients 118, 130,133, 147 and 154).

In two patients, mutations were related with both old and current therapies (patients 305 and 1,104). NNRTI resistance mutations were detected in plasma GRT after treatment interruptions in 3/46 (6%) patients receiving NNRTIs (Table 2). One major PI resistance mutation (V82A) was observed in 1 out of 8 (12.5%) patients treated with a PI (Table 2).

Evolution of resistance mutations in plasma and PBMC samples during the follow-up
Regarding the evolution of mutations on PBMC GRT and plasma GRT, the main findings were as follows. First, some major mutations that were not seen in the first PBMC GRT at baseline emerged in plasma GRT after TI (patients 101, 118, 121, 130, 133, 154 and 209). Second, the major NNRTI resistance-associated mutation K103N, observed at baseline as an archived mutation in one patient who was not receiving NNRTIs (patient 209), was not seen in subsequent GRTs on plasma performed after TI or on PBMCs performed after resumption of therapy. Third, in two patients (patients 133 and 154), NRTI resistance-related mutations detected in plasma GRT after TI seemed to be archived according to the previous treatment history, but they had not been detected in PBMC GRT at baseline. Fourth, in two patients (patients 130 and 147), resistance mutations detected in PBMC GRT at baseline were also observed in plasma GRT; and last, in two patients resistance mutations seen in plasma after TI were not found as archived in PBMCs in subsequent GRT performed after resumption of therapy (patients 133 and 209).

Virological failures
Virological failure occurred in three patients (patients 113, 124 and 305), all of them in the TI group (3/56, 5%). In all three patients, an NNRTI-containing regimen (two nevirapine and one efavirenz) had been interrupted. Two patients presented virological failure at months 27 and 36, after three and four treatment interruptions and resumptions, respectively, and no resistance mutations were found after TI in any of them (patients 113 and 124). In the other patient, who interrupted and restarted a nevirapine-containing regimen, one NNRTI resistance-associated mutation was observed after TI and virological failure occurred when the same HAART was resumed at month 12 (patient 305). Of note, in this patient, five NRTI resistance-associated mutations, related to both prior and current therapies, had also emerged after TI (Table 2). In these three patients, the grade of treatment adherence was assessed as >95% during the follow up. For supplementary information, see Additional file 2.

Discussion
Structured interruptions of ART in patients with undetectable plasma HIV-1 RNA have been associated with the emergence of resistance, mainly NRTI and NNRTI resistance-associated mutations. In our study, staggered interruption of NNRTIs 7 days before NRTIs resulted in lower rates of NNRTI-associated resistance mutations after treatment interruptions than has been reported in studies in which treatments were interrupted simultaneously; nonetheless, this strategy did not completely avoid this phenomenon. This approach has been examined in several studies, but the reported results are discordant [3,4,11–13]. It has been suggested that replacement of the NNRTI by a PI before ART interruption could reduce the risk of resistance in comparison with staggered interruption [3]. However, as has also been reported in other studies [3,6], we found that virological response can be achieved in some patients despite emergence of resistance mutations.

One interesting finding in our study is the dynamics of the mutational pattern in plasma RNA and PBMC
Table 1. Plasma and PBMCs HIV resistance mutations during treatment interruptions.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Previous HAART Treatment</th>
<th>Current HAART</th>
<th>Treatment Interruption</th>
<th>Initial Plasma</th>
<th>Initial PBMC</th>
<th>Second Plasma</th>
<th>Second PBMC</th>
<th>HAART Failure</th>
<th>Reasons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient A</td>
<td>AZT/ddI/3TC</td>
<td>AZT/ddI/3TC</td>
<td>E35D</td>
<td>50 copies/ml</td>
<td>No mutations</td>
<td>50 copies/ml</td>
<td>No mutations</td>
<td>Not available</td>
<td>NVP, nevirapine; PBMC, peripheral blood mononuclear cells; Prot, protease; RT, reverse transcriptase; SQV/r, ritonavir-boosted saquinavir; TDF, tenofovir disoproxil fumarate; TI, therapy interruption; 3TC, lamivudine.</td>
</tr>
</tbody>
</table>
provirial DNA over time. First, some of the mutations emerging in plasma after TI were related to the interrupted drugs, but others were related to previous therapies. Second, among patients with paired plasma and PBMC samples, concordance between proviral DNA and plasma RNA was observed in patients with no major mutations and only in one patient with a major mutation (patient 130). However, in the other patients with paired samples and major resistance-associated mutations in plasma HIV-1 RNA, the same mutations had not been observed in PBMCs at baseline. Furthermore, they were not found in PBMCs in subsequent genotyping. It is possible that these mutations were archived in proviral DNA as minority variants, which could not be detected [14,15], or they might have come from reservoirs other than PBMCs. Divergences between plasma RNA and proviral DNA genotypes after treatment interruption or failure have been described [16,17]. Resistance mutations usually emerge earlier in plasma RNA; hence, changes in the mutational archive in proviral DNA might not be observed for a lengthy period, despite emergence of resistance mutations in plasma HIV-1 RNA [18,19]. Lastly, HIV drug-resistant variants can revert to wild type in PBMCs earlier than in plasma [20].

Of the three patients who presented virological failure after treatment resumption, plasma RNA resistance

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Other drugs in current HAART</th>
<th>Previous antiretroviral treatments</th>
<th>GRT on PBMCs at baseline</th>
<th>Plasma GRT after TI</th>
<th>Resumption of ART (months)</th>
<th>Virological outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>118</td>
<td>TDF, ddi</td>
<td>AZT/3TC/IDV</td>
<td>No mutations</td>
<td>RT: M184V</td>
<td>No</td>
<td>–</td>
</tr>
<tr>
<td>121</td>
<td>AZT, 3TC</td>
<td>AZT/3TC/IDV</td>
<td></td>
<td>RT: K103N</td>
<td>Yes (15, 33)</td>
<td>Virological response</td>
</tr>
<tr>
<td>130</td>
<td>TDF, 3TC</td>
<td>AZT/3TC/IDV, d4T/3TC/IDV, d4T/3TC/EFV</td>
<td>NA</td>
<td>RT: D67N, K70R, K219Q</td>
<td>No</td>
<td>–</td>
</tr>
<tr>
<td>133</td>
<td>TDF, 3TC</td>
<td>AZT/3TC/IDV, d4T/3TC/EFV, d4T/3TC/NVP</td>
<td></td>
<td>RT: D67N, K70R, K219Q</td>
<td>Yes (3, 18)</td>
<td>Virological response</td>
</tr>
<tr>
<td>147</td>
<td>ddi, 3TC</td>
<td>AZT/3TC/IDV, d4T/3TC/NVP, ddi/3TC/NVP</td>
<td>RT: T69N, V118I</td>
<td>RT: T69N</td>
<td>Yes (9)</td>
<td>Virological response</td>
</tr>
<tr>
<td>154</td>
<td>ddi, 3TC</td>
<td>AZT/3TC/IDV, d4T/3TC/IDV, ddi/3TC/NVP</td>
<td>No mutations</td>
<td>RT: D67N, K70R</td>
<td>Yes (6)</td>
<td>Virological response</td>
</tr>
<tr>
<td>305</td>
<td>ABC, 3TC</td>
<td>AZT/3TC/IDV, d4T/3TC/IDV, d3TC/NVP</td>
<td>NA</td>
<td>RT: M41L, D67N, K70R, M184V, G190A, K219Q</td>
<td>Yes (3)</td>
<td>Virological failure</td>
</tr>
<tr>
<td>204</td>
<td>D4T, 3TC, IDV/r</td>
<td>AZT/3TC/IDV, d4T/3TC/IDV, d4T/3TC/NVP</td>
<td>NA</td>
<td>RT: M41L, M184V, T215Y</td>
<td>No</td>
<td>–</td>
</tr>
<tr>
<td>209</td>
<td>AZT, 3TC, SQV/r</td>
<td>AZT/3TC/IDV, d4T/3TC/IDV, d4T/3TC/EFV</td>
<td>RT: K103N, 1135T, Prot: L63P, V77I, I93L</td>
<td>RT: M184V</td>
<td>Yes (1, 9, 21)</td>
<td>Virological response</td>
</tr>
</tbody>
</table>

Mutations in bold are major mutations. Of note, two patients (113 and 124) in whom no resistance mutations had been observed during their follow-up presented virological failure after treatment resumption. Patient 113: previous treatments, zidovudine (AZT)/lamivudine (3TC)/indinavir (IDV) and stavudine (d4T)/didanosine (ddI)/efavirenz (EFV); current HAART, 3TC/ddI/EIDV; after four treatment interruptions virological failure was observed after the last resumption of therapy at month 36; K65R, L74V, M184V, K101E and G190S (reverse transcriptase [RT]) were detected in plasma RNA genotypic resistance test (GRT) at failure; no mutations had been detected in GRT on peripheral blood mononuclear cells (PBMCs) at baseline. Patient 124: previous treatments, AZT/3TC/ritonavir-boosted saquinavir (SQV); current HAART, AZT/3TC/EFV; resumption of therapy 12 months after treatment interruption resulted in, initially, good virological response (plasma HIV-1 RNA:50 copies/ml at month 21); rebound of plasma viral load was observed at month 24 and confirmed virological failure at month 27; M184V, 106A, 1135M and G190A (RT), and L10V and V77I (protease [prot]) were detected in plasma RNA GRT at failure; no mutations had been detected in GRT on PBMCs at baseline. See also Additional file 2. *Months since baseline. **12/24 (17%) patients, %22 (27%) patients. **Patient 305: plasma GRT after virological failure: RT: M41L, D67N, K70R, M184V, G190A, K219Q (the same mutation as in plasma GRT after treatment interruption). 2/8 (25%) patients. ABC, abacavir; ART, antiretroviral therapy; ddi, didanosine; ddd, stavudine; d4T, efavirenz; ddi, didanosine; d4T, boosted indinavir; NA, not available; NTV, nevirapine; PI, protease inhibitors; SQV, saquinavir; TDF, tenofovir disoproxil fumarate; TI, treatment interruption.

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mutations after TI had been seen in only one. Of note, these mutations were associated with both NRTIs and NNRTIs and were related to current therapy and to non-suppressive therapies received in the past. These findings indicate that plasma GRT associated with the previous treatment history are useful to assess the risk of virological failure in some patients with ART interruption and resumption, but a group of patients in whom failure cannot be predicted remains. As has been previously reported by other authors, PBMC GRT did not provide useful information for this purpose [21].

This study has some limitations. First, the sample size is small compared with other TI studies. In addition, some patients in whom mutations were observed after TI did not re-start ART (patients 101, 118, 204, 516 and 1,104) and so we were unable to determine the effect of these mutations on the risk of virological failure after treatment resumption. Some of these patients did not have an available GRT on PBMCs at baseline and we were unable to determine the presence of mutations in proviral DNA at baseline (patients 101, 204, 305, 516 and 1,104). Another limitation is the inability of our genotypic test to detect mutations in HIV variants present at a low frequency. Nonetheless, the availability of a subset of paired genotype samples from plasma HIV-1 RNA and from proviral DNA in PBMCs provided useful information about the dynamics of resistance after TI.

In summary, staggered interruption of NNRTIs 7 days before NRTIs did not avoid resistance emergence completely, but in most patients it did not necessarily lead to virological failure after treatment resumption. Plasma GRT and the history of previous ART regimens and treatment failures seem to be more useful than proviral DNA GRT to assess the risk of virological failure after treatment interruption and resumption, although this risk cannot be predicted in all patients. However, because some patients with resistance detected in plasma GRT after TI did not re-initiate therapy and some patients did not have a baseline GRT on proviral, these findings must be interpreted with caution.

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Disclosure statement

The authors declare no competing interests.

Additional files

Additional file 1: A list of STOPAR study group members can be found at http://www.intmedpress.com/uploads/documents/AVT-11-SC-2262_Imaz_Add_file1.pdf

Additional file 2: Detailed information about the follow-up of the patients can be found at http://www.intmedpress.com/uploads/documents/AVT-11-SC-2262_Imaz_Add_file2.pdf

References


