Temporary treatment during primary HIV infection does not affect virologic response to subsequent long-term treatment

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Abstract
Temporary combination antiretroviral therapy (cART) during primary HIV infection (PHI) did not affect the subsequent virologic response to long-term cART. Concerns for developing drug resistance mutations after TI have not been substantiated: even after interrupting early treatment, which was given during a period of 24 or 60 weeks shortly after HIV acquisition, our study patients still had a good response after subsequent reinitiation of long-term cART. This study contributes to the increasing data supporting temporary cART during PHI.
Introduction

In the Primo-SHM trial, a multicenter randomized trial comparing no treatment with 24- or 60-weeks of combination antiretroviral therapy (cART) during primary HIV infection (PHI), we recently demonstrated that temporary early cART lowered the viral setpoint and deferred the need for reinitiation of cART during chronic HIV infection [1]. Two other randomized studies also observed a modest delay in disease progression after a short course of cART in PHI [2, 3]. However, an important concern of temporary early cART, and of structured treatment interruptions (TI) in general, is the risk of developing drug resistance mutations after TI, especially in the case of NNRTI-based regimens, which compromise future treatment options.

The aim of this study was to assess the effect of temporary cART during PHI on the subsequent virologic response to long-term cART in patients who previously participated in the Primo-SHM trial. To this end, we compared the viral decay and the time to viral (re)suppression between the early treated patients who reinitiated cART and the patients in whom treatment was deferred until conventional criteria to start long-term cART had been reached.

Methods

Between May 2003 and March 2010 168 patients with laboratory evidence of PHI were randomized in the Primo-SHM trial to receive no treatment (naive patients, n=36) or 24 or 60 weeks of early cART (early treated patients, n=132) [4]. PHI was defined as a negative or indeterminate Western blot combined with a detectable plasma viral load (pVL), or, in case of a positive Western blot, a negative HIV screening test result ≤ 180 days. Early cART consisted of a quadruple triple-class regimen containing two NRTIs (zidovudine/lamivudine 300/150 mg bid), an NNRTI (efavirenz 600 mg qd) and a boosted PI (lopinavir/ritonavir capsules 533/133 mg bid). The latter was discontinued when the pVL had dropped <50 copies/ml. After January 2008 zidovudine/lamivudine was replaced by tenofovir/emtricitabine (245/200 mg qd) and lopinavir/ritonavir tablets (600/150 mg bid) replaced the capsules. Changes to this regimen were allowed in case of transmitted drug resistance or if one of the drugs was not tolerated. The study protocol required patients to reach a pVL <50 copies/ml before interrupting therapy as scheduled. Long-term cART was (re)started in case of two consecutive CD4 cell counts below 350 cells/mm³, severe constitutional symptoms, the occurrence of an AIDS defining event, or if the patient preferred on (re)initiating cART. Follow-up visits after (re)start of cART were scheduled according to standard treatment guidelines, i.e. after four weeks of treatment and every three months thereafter.

In the current study, we included the 94 out of 168 participants (56%) who had started or restarted long-term cART by September 2011 and who had at least one pVL measurement after (re)initiation of cART. (Re)Start regimens were at the discretion of the treating physician. Resistance testing was performed at diagnosis of PHI. To investigate possible acquired resistance during or after stopping of early cART, we performed resistance testing of the reverse transcriptase gene retrospectively in the first stored plasma sample with a pVL above 3.0 log₁₀ c/ml after TI, in the subset of early treated patients who were treated with an NNRTI
in the early phase and reinitiated long-term cART with a boosted PI. For the patients who were treated with an NNRTI during PHI and reinitiated with an NNRTI, we assumed that if NNRTI resistance had been acquired during early treatment, it would result in virological failure after restart with an NNRTI-based regimen. Data of the 24- and 60-weeks early treated patients were combined in all analyses because the viral decay was not significantly different between the two groups (data not shown). Sociodemographic characteristics and laboratory data at (re)start of long-term cART were compared between the naive and early treated patients using chi-square, Fisher’s exact and Kruskal-Wallis tests where appropriate.

Viral decay after start/restart of cART in naive/early treated patients was analysed using linear mixed models incorporating repeated measurements, which showed a tri-phasic pattern with distinct slopes from week zero to four, week four to eight and from week eight onwards. For this analysis patients were censored once they reached a pVL <50 copies/ml. A similar analysis was done for the early treated group, comparing viral decay during early initial cART with the decay after subsequent restart of cART. Time to viral (re)suppression, defined as a pVL <50 copies/ml, was compared between the two groups using Kaplan-Meier plots and multivariable Cox regression analysis. All analyses ignored modifications of treatment regimens, but censored patients at the moment of interruption of cART for more than two weeks. Data were analyzed using SAS version 9.2 (SAS institute, USA).

Results

Of the 36 naive and 132 early treated participants in the Primo-SHM trial, 31 (86%) and 63 (48%) had (re)initiated long-term cART by September 2011, respectively. In 52/63 early treated patients all antiretroviral drugs had been stopped simultaneously at TI: at that moment 31/63 (49%) were receiving dual-class NNRTI-based therapy, 15/63 (24%) dual-class boosted PI-based therapy, and 6/63 (10%) triple-class therapy. In the remaining 11/63 patients (17%) a staggered TI method was used, in which the NNRTI was stopped prior to the NRTI-backbone. Six early treated patients (6%) did not have a pVL <50 c/ml at TI (range 58-1882 copies/ml). The median time between TI and restart of long-term cART was 1.9 (IQR 0.9-3.1) years.

89/94 (re)starting participants (95%) were men. Mean age and CD4 count at (re)start were 44 (SD 9) years and 290 (110) cells/mm³, respectively, and were not significantly different between the naive and early treated patients. The naive patients had a higher mean pVL at (re)start (5.0 (SD 0.7) versus 4.7 (0.7) log₁₀ c/ml; P=0.07) and less transmitted drug resistance mutations at the moment of PHI-diagnosis (0 versus 8 (14%); P=0.048). Naive patients initiated long-term cART more often with an NNRTI-containing regimen than early treated patients (24 (77%) versus 37 (59%); P=0.10). Four naive (13%) and 23 early treated patients (37%) (re)started long-term cART with a boosted PI (P=0.03), and three (10%) versus three (5%) patients, respectively, (re) initiated with triple-class therapy (P=0.40).

Drug resistance testing was performed after TI in 20/23 early treated participants who had been treated with an NNRTI and restarted long-term cART with a boosted PI. None of these patients had developed a drug resistance mutation. Of the remaining three patients, one harboured a 103N mutation, which was already present at diagnosis of PHI (this patient had
Viral resuppression after early cART started with the standard quadruple triple-class regimen before baseline resistance testing results were known, once results were available the regimen was adapted and the NNRTI was removed), and for two patients no stored samples were available. The median interval between TI and the timepoint of resistance testing was 4 (IQR 3.7-8) weeks. Other patient characteristics (transmission route, ethnicity, history of CDC-events, virus subtype) were comparable between the two groups. One early treated patient was lost-to-follow-up after restart of long-term cART and he was censored at this visit. Patient characteristics are summarized in Table 1.

Table 1. Patient characteristics at (re)start of treatment

<table>
<thead>
<tr>
<th></th>
<th>Total (N=94)</th>
<th>No treatment during PHI (N=31)</th>
<th>Early cART during PHI (N=63)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years), mean (SD)</td>
<td>44.9 (9)</td>
<td>44.1 (10)</td>
<td>43.5 (8)</td>
<td>0.9</td>
</tr>
<tr>
<td>Men</td>
<td>89.95%</td>
<td>31 (100)</td>
<td>58.92%</td>
<td>0.2</td>
</tr>
<tr>
<td>MSM</td>
<td>78.83%</td>
<td>26 (84)</td>
<td>52.83%</td>
<td>0.9</td>
</tr>
<tr>
<td>Born in the Netherlands</td>
<td>84.89%</td>
<td>28 (90)</td>
<td>56.89%</td>
<td>1.0</td>
</tr>
<tr>
<td>History of CDC C-event</td>
<td>11.12%</td>
<td>2 (7)</td>
<td>9.14%</td>
<td>0.3</td>
</tr>
<tr>
<td>CD4 count (cells/mm³), mean (SD)</td>
<td>290 (110)</td>
<td>273 (133)</td>
<td>299 (96)</td>
<td>0.3</td>
</tr>
<tr>
<td>Plasma HIV-1 RNA (log₁₀ copies/ml), mean (SD)</td>
<td>4.8 (0.7)</td>
<td>5.0 (0.7)</td>
<td>4.7 (0.7)</td>
<td>0.07</td>
</tr>
<tr>
<td>Subtype B virus a</td>
<td>75 (88%)</td>
<td>25 (89%)</td>
<td>50 (88%)</td>
<td>1.0</td>
</tr>
<tr>
<td>Genotypic resistance mutations at diagnosis of PHI a</td>
<td>8 (9%)</td>
<td>0 (0)</td>
<td>8 (14)%</td>
<td>0.048</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Initiation of cART during chronic HIV-infection</th>
<th>Total (N=94)</th>
<th>No treatment during PHI (N=31)</th>
<th>Early cART during PHI (N=63)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>- triple-class therapy</td>
<td>6 (6)</td>
<td>3 (10)</td>
<td>3 (5)</td>
<td>0.4</td>
</tr>
<tr>
<td>- dual-class NNRTI</td>
<td>61 (65)</td>
<td>24 (77)</td>
<td>37 (59)</td>
<td>0.1</td>
</tr>
<tr>
<td>- dual-class PI</td>
<td>27 (29)</td>
<td>4 (13)</td>
<td>23 (37)</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Data are n (%) unless indicated otherwise. cART, combination antiretroviral therapy; MSM, men who have sex with men; (N)NRTI, (non-)nucleoside reverse transcriptase inhibitor; PHI, primary HIV infection; PI, boosted protease inhibitor.

a 9 missing patients: 3 in the non-early treated and 6 in the early treated group.

b 5 patients carried a M41L mutation, 2 patients a M46I mutation of whom one also had a T215S mutation and one carried a K103N mutation.

All naive and early treated patients achieved viral (re)suppression. Viral decay after treatment (re)initiation was similar between the naive and early treated patients: during the first four weeks the pVL decreased with 0.62 and 0.58 log₁₀ copies/ml/week respectively (P=0.32), from week four to eight with 0.087 and 0.13 log₁₀ copies/ml/week (P=0.37), and from eight weeks onward with 0.043 and 0.027 log₁₀ copies/ml/week (P=0.23) (Figure 1A). Adjusting the viral decay for the difference in pVL at (re)start of long-term cART also showed no significant differences between the two groups (data not shown). The median time to viral (re)suppression in naive and early treated patients was 16.4 (IQR 9.6-20.6) and 16.6 (8.7-21.0) weeks, respectively (log-rank, P=0.72). In the Cox analysis early treatment during PHI as compared to no treatment was not associated with time to viral resuppression (HR 0.81 (95% CI 0.27-2.39); P=0.70). As expected, a higher vireamia at (re)start of long-term cART was predictive for a longer time to viral suppression (HR 0.37 per 1 log₁₀ copies/ml increase (95% CI 0.25-0.56; P<0.001). Other
Viral resuppression after early cART

parameters, including reinitiating with an NNRTI-based regimen or with triple-class therapy, were not associated with time to viral (re)suppression (data not shown).

For the early treated group, we additionally compared viral decay during the early treatment episode and subsequent restart of long-term cART (Figure 1B). During the first four weeks the pVL decreased with 0.52 and 0.57 log_{10} copies/ml/week, respectively (P=0.16), from week four to eight with 0.13 and 0.14 log_{10} copies/ml/week (P=0.70), and from eight weeks onward with 0.048 and 0.021 log_{10} copies/ml/week (P=0.003).

Discussion

Temporary cART during PHI was not associated with a diminished virologic response after subsequent reinitiation of long-term cART, when we compared early treated with naive patients and when we compared both treatment episodes in early treated patients. This suggests that temporary early cART did not select for clinically relevant drug resistance and supports the use of early treatment during PHI. Of note, the slower decline of the pVL from 8 weeks onward in...
the second treatment period of the early treated patients is probably an artefact of the model, because patients had a lower baseline pVL at restart than during early treatment and were usually already undetectable by week 8.

We did not perform resistance testing after TI in all our patients. We therefore cannot exclude with certainty that there might have been selection of drug resistance after TI. This is in particular relevant for patients who had interrupted an NNRTI-based regimen, because of the long half-life of NNRTIs. However, in all patients, irrespective of the regimen, the pVL was resuppressed upon restart, which virtually excludes clinically important mutations. Early treated patients reinitiated long-term cART more often with a boosted PI than naive patients, which may have overcome possible acquired NNRTI mutations. We therefore retrospectively performed genotypic resistance testing after TI in 20/23 of these patients and did not detect any new drug resistance mutation. The reason for the preference of a PI-containing regimen was usually that patients preferred not to restart an NNRTI because of side effects they had experienced previously during the early cART period. Therefore, there is no indication of acquired resistance in our patients.

Our study is supported by another study in which 37 PHI-patients were treated with temporary early cART and no drug resistance was observed after TI [5]. However, in this study the NNRTI was stopped 96h before the NRTI-backbone. Because NNRTIs have a slower metabolism and a low genetic barrier to resistance, simultaneous TI of an NNRTI-containing regimen may result in a period of NNRTI-monotherapy, which may select for drug resistance mutations [6]. NNRTI-drug resistance mutations that were selected after intrapartum exposure to single-dose nevirapine in HIV-infected women have been associated with decreased virologic response after subsequent treatment with an NNRTI-containing regimen [7, 8]. Noteworthy, the pVL in these women exposed to single-dose nevirapine was much higher than the pVL in a controlled TI-setting in which patients have an undetectable pVL. In the SMART trial [9], NNRTI-drug resistance mutations were more common in case of simultaneous TI than in case of a staggered or a switched interruption, in which the NNRTI is replaced by a boosted PI [10]. However, in SMART most drug combinations included a zidovudine/lamivudine-backbone in combination with an NNRTI [9], whereas in our trial half of the patients were using a tenofovir-containing regimen, which has a longer half-life [11], and together with an NNRTI forms a more balanced regimen that is less prone to development of drug resistance when treatment is discontinued simultaneously. To date, there is no clear consensus how to stop cART regimens [12]. In our study we found no indication for selection of drug resistance after interrupting all drugs simultaneously.

In conclusion, temporary cART during PHI was not associated with a reduced virologic response after subsequent reinitiation of long-term cART. Concerns for developing drug resistance mutations after TI have not been substantiated: even if patients interrupt early treatment, they still have a good response after subsequent reinitiation of cART. This study contributes to the increasing data supporting temporary cART during PHI.
Acknowledgments


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Contributors

MLG and JMP established the cohort and together with SJ, FPK, EFS and PK, they were responsible for patient enrolment and trial conduct at each study site. LG assisted with the data retrieval and MLG and FWNMW conducted the statistical analysis. MLG drafted the manuscript and JMP and JL critically revised the manuscript. All authors reviewed and approved the final version of the manuscript.
References


