Drug resistance mutations 18 months after discontinuation of nevirapine-based ART for prevention of mother-to-child transmission of HIV in Malawi

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Objectives: The objective of this study was to determine the prevalence of drug resistance mutations among HIV-positive women in Malawi 18 months after discontinuing nevirapine-based ART for the prevention of mother-to-child transmission.

Patients and methods: HIV-infected antiretroviral-naive (except for single-dose nevirapine) pregnant Malawian women receiving a nevirapine-based triple antiretroviral regimen from Week 25 of gestation until 6 months of breastfeeding were included in this analysis. Drug resistance was assessed in HIV-DNA 24 months post-partum and at baseline (before the initiation of treatment). In patients with resistance, the presence of mutations was also evaluated in the corresponding plasma samples.

Results: Seven out of 42 (16.7%) women studied had archived drug resistance at Month 24 [six cases had NNRTI-associated mutations and two cases the M184I mutation]. In four cases, resistance mutations were already present at baseline (all NNRTI mutations). In three cases, there was an emergence of ‘new’ resistance (also present in the plasma in one case). Of the 35 women without resistance mutations at Month 24, only one subject had resistance mutations at baseline. Baseline resistance was significantly more common among women with mutations at 24 months compared with those harbouring a WT virus (4/7 versus 1/35, P<0.001).

Conclusions: Among women who had discontinued drugs 6 months post-partum, only 3/42 (7.1%) had accumulated new resistance mutations in HIV-DNA 2 years after delivery. These findings are reassuring in terms of the safety of the Option B strategy for the prevention of HIV mother-to-child transmission.

Introduction

According to the most recent guidelines of the WHO, both life-long antiretroviral administration irrespective of the CD4+ count (Option B-Plus) and the administration of triple combination therapy until the risk of vertical transmission has ceased (Option B) are recommended for preventing the mother-to-child transmission of HIV.1 The risks and benefits of the two options should be evaluated to define future recommendations. One of the possible drawbacks associated with the Option B strategy is the emergence of drug resistance as a consequence of the interruption of drug treatment.2 The frequency of emergence of mutations is variable according to the regimens used, the interruption strategy (simultaneous versus staggered) and the timing of the resistance analysis.3 – 6 This last issue is relevant since, in the absence of drug pressure, mutations may soon no longer be detectable in plasma even though they may be archived in the HIV-DNA of latently infected cells. Since women who have discontinued drug treatment are candidates for restarting ART when reaching the criteria for treatment, these archived mutations may be important in patients’ responses to ART.

The present study, conducted in HIV-infected pregnant women enrolled in a study of ART administration under the Option B approach, and receiving a nevirapine-based triple combination regimen until 6 months post-partum, had the following objectives: (i) to determine the prevalence of resistance-associated mutations in HIV-DNA 18 months after discontinuation of treatment; (ii) to define the prevalence of pre-existing resistance in the baseline (pre-treatment) samples; and (iii) to evaluate the determinants of the emergence of resistance.

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Patients and methods

Study population

The study population included treatment-naïve (with the exception of single-dose nevirapine) HIV-infected pregnant women in Malawi who were enrolled in an observational study that aimed to assess the safety and pharmacokinetics of maternal ART administration during breastfeeding [the Safe Milk for African Children (SMAC) study].7,8 The study was conducted within the Drug Resource Enhancement against AIDS and Malnutrition (DREAM) programme of the Community of S. Egidio and received ethical approval from the National Health Research Committee in Malawi (Approval Number 486). A separate informed consent form was signed by all participants.

Between February 2008 and February 2009, the study enrolled HIV-positive pregnant women who received, in the case of a CD4+ cell count >350 cells/mm³, a regimen consisting of zidovudine, lamivudine and nevirapine from Week 25 of gestation until 6 months post-partum (the recommended duration of breastfeeding at the time of the study). At the point of drug interruption, a 3 week tail of zidovudine and lamivudine was administered. Women experiencing nevirapine-associated toxicity were allowed to substitute this drug with lopinavir/ritonavir.

Virological analyses

Plasma and whole blood samples were stored frozen at −80°C after collection and then shipped in dry ice to the laboratory of the Istituto Superiore di Sanità where they were analysed. The viral load was measured using the Versant kPCR assay (Siemens Healthcare Diagnostics, Deerfield, IL, USA). The presence of drug resistance was first assessed in HIV-DNA both at baseline and at Month 24, and then, for those testing positive, in the corresponding plasma samples. Resistance was assessed by the use of the TruGene Genotyping kit (Siemens Healthcare Diagnostics) after the extraction of proviral DNA from whole blood (QIAamp DNA Blood Mini Kit, Qiagen, Hilden, Germany). Resistance mutations were classified according to the International AIDS Society–USA Panel (2014).

Data analysis

Results are presented as medians with IQRs, and proportions. Quantitative variables were compared using the Mann–Whitney test and categorical data were compared using the χ² or Fisher test, as appropriate. Statistical analysis was performed using SPSS, version 22.0 (IBM, Somers, NY, USA).

Results

Study population

Of the 99 women enrolled in the study who had discontinued ART 6 months after delivery and had reached 2 years of follow-up, 21 had restarted treatment either because of a new pregnancy or because of meeting the criteria for treatment; 78 were not receiving ART and had HIV-RNA >50 copies/mL. Blood samples were available both at Month 24 and at baseline (before the initiation of treatment) for 47 women of the latter group, who were therefore included in the study. Of the 47 women with available samples, sequences at Month 24 were obtained for 42. The characteristics of the women who had sequences available are reported in Table 1. Their baseline CD4+ cell count was 504 cells/mm³, and 6 months post-partum 79% of the women had HIV-RNA <50 copies/mL. Four women switched to lopinavir/ritonavir during pregnancy because of nevirapine-associated toxicity.

<table>
<thead>
<tr>
<th>Table 1. Characteristics of patients included in the study</th>
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<tbody>
<tr>
<td>Number</td>
</tr>
<tr>
<td>Age (years), median (IQR)</td>
</tr>
<tr>
<td>WHO Stage 1, n (%)</td>
</tr>
<tr>
<td>WHO Stages 2–4, n (%)</td>
</tr>
<tr>
<td>Parity, median (IQR)</td>
</tr>
<tr>
<td>Baseline CD4+ count (cells/mm³), median (IQR)</td>
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<tr>
<td>Baseline plasma HIV-RNA (log₁₀ copies/mL), median (IQR)</td>
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<tr>
<td>ART duration during pregnancy (days), median (IQR)</td>
</tr>
<tr>
<td>Month 6 CD4+ count (cells/mm³), median (IQR)</td>
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<tr>
<td>Month 6 HIV-RNA (log₁₀ copies/mL), median (IQR)</td>
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<tr>
<td>Month 6 HIV-RNA &lt;50 copies/mL, percentage of patients</td>
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<tr>
<td>Month 24 CD4+ count (cells/mm³), median (IQR)</td>
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<td>Month 24 HIV-RNA (log₁₀ copies/mL), median (IQR)</td>
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Resistance analysis

Seven patients out of 42 (16.7%) had resistance-associated mutations in their HIV-DNA at Month 24 (Table 2). Five women had only NNRTI-associated mutations, one only the M184I mutation, and one both the M184I and M230I mutations. In five out of seven cases, the mutations were present also in the corresponding plasma samples (all patients who had only NNRTI resistance). None of the patients with mutations had switched to a PI-based regimen. In four cases, the same mutations were found in the HIV-DNA of the baseline samples. In one case, the mutation was present during treatment (at Month 6), while in two cases (involving the M184I mutation) there was no mutation in the baseline or on-treatment samples. Therefore in 3 cases out of 42 (7.1%), the strategy was associated with the emergence of ‘new’ resistance. Baseline resistance was found in 1 woman (harbouring the K103N mutation) out of 35 with no resistance at Month 24. The prevalence of baseline resistance was significantly different between the women who had or did not have resistance at Month 24 (4/7 versus 1/35, P<0.001). No other difference was found between women with or without resistance.

Discussion

In this study, we assessed the prevalence of resistance mutations 2 years after delivery in HIV-positive women who had discontinued antiretroviral drugs 6 months post-partum, according to the recommendations for the duration of breastfeeding at the time of the study. Under the Option B approach, HIV-positive pregnant women not meeting the criteria for treatment are allowed to suspend antiretroviral drugs when the risk of transmission to their infants has ceased. Drug discontinuation may favour the emergence of drug resistance, and several groups including ours have reported variable prevalences of drug resistance at the discontinuation of treatment.3–6 However, assessing drug resistance only at treatment discontinuation may have some limitations including
sample availability and the timing of testing, since early testing is often not feasible because of the low viral load and late testing may be falsely negative since mutations may rapidly disappear in the absence of drug pressure. In this study, we analysed archived mutations in HIV-DNA 2 years post-partum in order to gain a more complete picture of the resistance burden of these patients. In patients who have interrupted treatment, drug-resistant mutants that are not replicating at a high level may persist as archived resistance in the HIV-DNA of latently infected cells and may affect the response to subsequent ART.9 – 11

In our study, drug resistance was present in 16.7% of the patients studied at Month 24. However, it has to be emphasized that the majority of the mutations found (four out of the seven cases, all NNRTI mutations) were present in the baseline samples, possibly reflecting either previous exposure to single-dose nevirapine or, in the cases of the E138A mutation, a possible polymorphism for this mutation. In one further case involving NNRTI mutations, the K103N mutation was already present at the point of drug discontinuation since this patient did not show viral suppression 6 months post-partum. Interestingly, while the NNRTI mutations at Month 24, besides being archived in the HIV-DNA, were also present in the corresponding plasma samples, there was in the women with the M184I archived mutations a WT virus in the plasma samples, in agreement with the rapid loss observed for this mutation.12 Overall, therefore, a true ‘new’ emergence of resistance associated with this strategy was seen in 7.1% (3/42) of the patients, suggesting that a good response to the reinitiation of treatment can be expected in the majority of patients. Among the women not harbouring resistant strains at Month 24, only one had baseline resistance, a significantly lower proportion than in women with resistance at Month 24 (1/35 or 2.9% versus 4/7 or 57.1%). The overall proportion of patients with baseline resistant strains (11.9%, 5 out of 42) is higher than in previous findings for therapy-naive pregnant women in Malawi,13 probably due to possible previous exposure to single-dose nevirapine in this cohort; if the E138A mutation was excluded, this proportion would decrease to 7.1%, which is similar to the previously reported prevalence of resistance of 6%.

The limitations of our study include a relatively small sample size and the use of population sequencing. The use of sensitive sequencing methods would probably have increased the prevalence estimates of resistance mutations, although the role and possible impact of minority variants at the reinitiation of treatment are still under debate.14 – 17 Further, we assessed the emergence of resistance in a population receiving a nevirapine-based regimen, while the currently recommended first-line treatment in resource-limited settings1 is efavirenz based. However, the emergence of resistance at discontinuation of treatment has been shown to be similar2,18 for these two drugs, which belong to the same class and share a similar genetic barrier.

A growing number of countries are now planning to implement the Option B-Plus approach; however, for those countries that consider the adoption of this approach not sustainable, these findings are reassuring.

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Transparency declarations
S. V. has received honoraria from ViiV, Gilead and Merck for scientific board membership. All other authors: none to declare.

Author contributions
L. P. conceived the study and participated in its design and coordination, C. M. G., R. L. and R. A. were responsible for the laboratory assays, M. A. was involved in the design and the conduct of the study, G. L. and S. M. contributed to the data collection and management, H. J. and J.-B. S. were responsible for the clinical care of the enrolled patients, M. C. M. and S. V. participated in the design of the study and critically revised the manuscript, and M. G. was involved in the design and the conduct of the study, performed the statistical analysis and drafted the manuscript.

References