AIDS Rev 2002;4:59-63

Nevirapine Resistance after Single Dose Prophylaxis

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Abstract

Nevirapine (NVP) is a potent non-nucleoside inhibitor of HIV-1 reverse transcriptase. In 1999, the HIVNET 012 trial in Uganda demonstrated that a simple regimen of NVP prophylaxis can dramatically reduce the rate of HIV-1 mother-to-child transmission (MTCT). In the HIVNET 012 regimen, women received a single dose of NVP in labor, and infants received a single dose of NVP within 72 h of birth. The simplicity, efficacy, and low cost of the HIVNET 012 regimen are attractive for prevention of MTCT in resource-poor settings. Plans are underway to implement this regimen in several resource-poor countries. Single mutations in HIV-1 RT can cause high level NVP-resistance and are likely to exist in most HIV-1 infected patients at low levels prior to antiretroviral drug exposure. This favors emergence of NVP-resistant HIV-1 following NVP exposure. NVP-resistant HIV-1 has been shown to emerge in some women and infants following single dose NVP. Emergence of NVP-resistant HIV-1 in this setting is more common among women with high baseline viral loads and low baseline CD4 cell counts. The rate of NVP-resistance in women receiving single dose NVP prophylaxis may also be influenced by HIV-1 subtype. The NVP-resistant HIV-1 typically fades from detection in women and infants over time. We review studies examining the emergence and fading of NVP-resistant HIV-1 in women and infants who received single dose NVP prophylaxis, and discuss the potential clinical relevance of NVP-resistance in this setting.

Key words

Nevirapine. Prophylaxis. HIV-1. Resistance. Mother-to-child transmission.

Introduction

HIV-1 mother-to-child transmission (MTCT) occurs in 14-40% of pregnancies without intervention, accounting for most cases of pediatric AIDS. Millions of infants have been infected with HIV-1 worldwide. Potent combinations of antiretroviral drugs are available to prevent HIV-1 MTCT. Unfortunately, cost and other factors have limited the availability of antiretroviral prophylaxis in many countries. This is particularly true in developing countries, which shoulder the greatest burden of the HIV-1 epidemic. Recent discovery of effective, less expensive regimens for prevention of MTCT has brought new hope for reducing the rate of HIV-1 MTCT around the world.

There are 3 classes of antiretroviral drugs currently available for clinical use: protease inhibitors (PIs), nucleoside and nucleotide reverse transcrip-

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tase (RT) inhibitors (NRTIs), and non-nucleoside RT inhibitors (NNRTIs). The 3 licensed NNRTIs include nevirapine (NVP), delavirdine (DLV), and efavirenz (EFV). The pharmacologic properties of NVP suggested that it might be effective in preventing MTCT as a single dose regimen. NVP is rapidly absorbed after oral administration, rapidly crosses the placenta, and has a long half-life in pregnant women and infants[1,2].

In 1999, the Ugandan HIVNET 012 trial compared the efficacy of a single dose NVP regimen and a short course of zidovudine (AZT) prophylaxis for prevention of MTCT[3,4]. Women in the NVP arm received a single 200 mg oral dose of NVP at the onset of labor, and infants received a single 2 mg/kg oral dose of NVP within 72 h of birth. Women in the AZT arm received 600 mg AZT orally at onset of labor, then 300 mg every 3 h until delivery and infants received 4 mg/kg AZT orally twice daily for 7 days after birth. All of the women enrolled in HIVNET 012 were antiretroviral drug *naïve*, and none received antiretroviral therapy after receiving AZT or NVP prophylaxis, consistent with the standard of care in Uganda. The estimated risk of transmission at 14-16 weeks of age was 25.1% in the AZT group, but only 13.1% in the NVP group. The NVP regimen was 47% more effective than the AZT regimen and was considerably less expensive.

The simplicity, efficacy, and low cost of the HIVNET 012 regimen are attractive for prevention of MTCT in resource-poor settings. This regimen was recently recommended by the World Health Organization as an option for prevention of MTCT[5]. Plans are underway to implement the HIVNET 012 regimen in several resource-poor countries. In the United States (US), the regimen is also recommended for prophylaxis in women who did not receive antiretroviral drugs during pregnancy[6]. If this regimen were rapidly and widely implemented, it could protect millions of infants from HIV-1 infection in the next decade.

Emergence of drug resistance following single dose NVP

The potential impact of antiretroviral prophylaxis on reducing the number of children infected with HIV-1 should not be underestimated. However, a concern to global health is that use of antiretroviral drugs in this setting will increase the overall prevalence of drug resistant HIV-1. This is of particular concern in resource-poor settings, where shorter, 1- or 2-drug prophylactic regimens are less likely to fully suppress HIV-1 replication. Single mutations in HIV-1 RT (e.g. K103N and Y181C) can cause high level NVP-resistance and are likely to exist in most patients at low levels prior to antiretroviral drug exposure. In past years, when NVP was provided as monotherapy for treatment of HIV-1 disease, NVP-resistant HIV-1 was rapidly selected[7]. Below, we review studies examining the emergence and fading of NVP-resistant HIV-1 following single dose NVP prophylaxis in the setting of MTCT.

Background on HIV-1 subtypes

Most HIV-1 viruses can be categorized into subtypes (clades) based on genetic differences. To date, almost all of the information characterizing HIV-1 drug resistance mutations and their effects on drug susceptibility comes from studies of subtype B, the most common subtype in the U.S. and Europe[8]. However, the overwhelming majority of HIV-1 infections worldwide are caused by other subtypes. Research on drug resistance in cohorts with non-subtype B HIV-1 is becoming increasingly important for two reasons: 1) the prevalence of non-subtype B is increasing in the U.S. and other regions where antiretroviral drugs are widely used, and 2) the availability and use of antiretroviral drugs is growing throughout the world, where most infections are caused by non-B HIV-1. The single dose NVP prophylaxis regimen is most likely to be introduced into regions where non-B HIV-1 is prevalent. In Uganda, where the HIVNET 012 trial was performed, most HIV-1 infections are caused by subtypes A and D[9,10], although infection with other subtypes (e.g. C, G, recombinant HIV-1) also occurs.

NVP-resistance mutations

To date, NVP-resistance mutations have been characterized almost exclusively in subtype B HIV-1. Several mutations are associated with high-level resistance (e.g. K103N, V106A, Y181C/I, Y188C/H/L, G190A/S, M230L) while others are associated with low-level resistance (e.g. A98G, L100I, K101E, V108I, V179D); accessory mutations (e.g. K101Q, V106I, P225H, Y318F) and mutations causing hypersusceptibility (e.g. P236L) have also been described [Stanford HIV RT and protease Sequence Database: http://hivdb.stanford.edu/cgi-bin/NNRTIResiNote.cgi]. Deletion of RT codon 69 may also confer resistance[11]. In addition, amino acid substitutions at positions 135 and 283 in HIV-1 RT confer resistance to NNRTIs, with up to 14-fold range in mean susceptibility to NVP and DLV[12]. It is not known whether the effects of these mutations are the same or different in other HIV-1 subtypes.

NVP-resistance mutations in HIV-1 from Uganda

Most available studies of NVP-resistance following single dose NVP prophylaxis were performed in women and infants from Uganda (see below). To interpret these studies, it is important to determine whether mutations associated with NVP-resistance can be detected in HIV-1 from Ugandan patients prior to NVP administration. Available studies suggest that NVP-resistance mutations are infrequent in antiretroviral drug *naïve* adults from Uganda. Analysis of 27 antiretroviral drug *naïve* Ugandan adults[13], and of nine women in HIVNET 012 prior to NVP administration[14], revealed no primary NVPR mutations. Two other studies, one with 11 and one with

14 antiretroviral drug *naïve* Ugandan adults, also failed to find any primary drug resistance mutations[15,16]. Interestingly, the 283I mutation was identified in 11/17 drug *naïve* Ugandan adults with subtype A and 2/10 Ugandan with subtype D[13]. The combination of mutations, 135T and 283I, which was associated with a significant decrease in susceptibility to all three NNRTIs[12], was present in one patient in that cohort[13]. It is not known whether those polymorphisms influence the natural susceptibility of HIV-1 to NVP or the probability that NVP-resistance will arise following single dose NVP prophylaxis.

Emergence and fading of NVP-resistance in women following single dose NVP

Selection of NVP-resistance after single dose NVP prophylaxis was first studied in the Ugandan Phase I/II trial, HIVNET 006. In HIVNET 006, 21 women received the same single dose NVP regimen that was subsequently given to women in HIVNET 012[2]. HIV-1 with the K103N NVP-resistance mutation was detected in 3/15 (20%) women 6-8 weeks after delivery (6-8 weeks after NVP administration)[17]. NVP pharmacokinetics were also studied in HIVNET 006. Interestingly, women who had the K103N mutation detected 6-8 weeks post NVP had a longer median NVP elimination half-life, decreased median oral NVP clearance, and increased median area under the NVP concentration curve[17]. Those findings suggested that prolonged maternal exposure to NVP favored emergence of HIV-1 variants with the K103N mutation.

Selection NVP-resistance after single dose NVP prophylaxis was further examined in the larger cohort of women and infants enrolled in the Ugandan HIVNET 012 trial described above[14]. In HIVNET 012, NVP-resistance mutations were detected in 21/111 (19%) of women 6-8 weeks after NVP administration. Similar rates of resistance were observed among women whose infants were and were not infected. The NVP-resistance mutations were not detected in baseline (pre-NVP) samples, indicating that the NVP-resistant variants were selected following NVP administration. In this cohort, emergence of NVP-resistance was associated with a higher baseline viral load and lower baseline CD4 cell count[14]. The NVP-resistant variants appeared to fade in women over time. By 12-24 months after delivery, the NVP-resistance mutations were no longer detectable.

Emergence and fading of NVP-resistance in infants following single dose NVP

In HIVNET 012, 37 infants were HIV-1 infected by age 6-8 weeks, despite NVP prophylaxis. The majority of those infants had evidence of HIV-1 infection at the time of birth. Among the 24 infants who had samples available for analysis, NVP-resistance mutations were detected in 11 (46%) of the infants at 6-8 weeks of age[14]. The NVP-resistance mutations were not detected in pre-NVP (birth) samples from the infants. The NVP-resistance mutations also faded from detection in infants over time. In many cases, the NVP-resistance mutations were no longer detectable by 14-16 weeks of age.

Ninety-eight percent of women in HIVNET 012 breastfed their infants. NVP-resistance was also examined in infants who were diagnosed with HIV-1 infection after age 6-8 weeks, who were presumably infected with HIV-1 through breast-feeding[14]. Only 1 of 9 infants with late HIV-1 infection had evidence of NVP-resistance.

Comparison of NVP-resistance mutations in HIV-infected infants and their mothers

Interestingly, different patterns of NVP-resistance mutations were detected in women and infants in HIVNET 012 6-8 weeks after NVP administration. In women, the most common mutation was K103N; in infants it was Y181C (Table 1)[14,18]. Furthermore, in each case where both the mother and infant had NVP-resistance 6-8 weeks after delivery, the pattern of NVP-resistance mutations was different[14]. The mechanisms responsible for emergence of different patterns of NVP-resistance mutations in women *vs.* infants are not known. Factors such as viral load, the level of NVP exposure, and the prevalence of pre-existing variants with NVP-resistance mutations may be important.

The emergence of variants with K103N *vs* Y181C is relevant for two reasons: In subtype B, 1) the level of NVPR is typically higher for variants with Y181C *vs* K103N[19], and 2) variants with K103N are cross-resistant to both of the other licensed NNRTIs, DLV and EFV, whereas variants with Y181C are cross-resistant to DLV, but retain susceptibility to EFV[19]. This issue of cross-resistance has not been explored in other subtypes. It will be important to evaluate the cross-resistance of non-subtype B variants with K103N or Y181C to DLV and EFV, since this information could theoretically be used to select drugs for treatment of women and infants with non-subtype B who develop NVPR following NVP prophylaxis. Caution is warranted, however, since two recent studies in subtype B suggest that patients

Table 1. *Comparison of the frequency of K103N and Y181C NVP-resistance mutations in women vs infants 6-8 weeks following single dose NVP prophylaxis in HIVNET 012*

	Women	Infants
K103N*	16/18 (89%)	2/11 (18%)
Y181C*	6/18 (33%)	9/11 (82%)

*alone or in combination with other mutations

who have failed NVP may not respond clinically to EFV, even if Y181C is the only mutation detected[20,21].

Emergence and fading of NVP-resistance following single dose NVP in women with different HIV-1 subtypes

The HIVNET 012 offered a unique opportunity to examine the impact of subtype on emergence of antiretroviral drug resistance for several reasons: 1) most women in Uganda have either subtype A or D HIV-1 infection, allowing a comparison of drug resistance in these two subtypes in a single cohort; 2) all women in the trial were antiretroviral drug *naïve* prior to NVP administration; 3) all women in the trial received the same single dose NVP regimen; 4) plasma samples were collected from all women at defined time points following NVP administration which were available for genotypic analysis of HIV-1, and 5) a large data base of clinical and laboratory data was available for this cohort, allowing examination of co-variates such as baseline viral load and baseline CD4 cell count that may influence emergence of drug resistance.

In HIVNET 012, a higher proportion of women with subtype D developed NVP-resistance compared to women with subtype A[22]. Similar NVP-resistance mutations were detected in women with these two subtypes. The difference in the rates of NVP-resistance in women with subtype A *vs* D did not appear to reflect a difference in the stage of disease, since the baseline viral loads and baseline CD4 cell counts were similar in the two groups[22].

The finding of a higher rate of NVP-resistance in women with subtype D 6-8 weeks after single dose NVP prophylaxis could reflect more frequent selection of NVP-resistant variants, or more sustained circulation of those variants in women following delivery. Further studies are needed to evaluate the relative fitness of subtype A *vs* D with NVP-resistance mutations, and to compare the kinetics of emerging and fading of those variants in women receiving NVP prophylaxis.

Emergence of NVP-resistance after single dose NVP prophylaxis was also examined in HIVNET 023, a Phase I/II trial performed in South Africa and Zimbabwe where subtype C predominates[23]. Women received the same NVP regimen as in HIVNET 012. HIV genotyping was performed for 30 women from HIVNET 023 with subtype C HIV-1 using samples collected 8 weeks after delivery (8 weeks after single dose NVP). Data from HIVNET 023 was combined with data from HIVNET 012 (including 48 women with subtype A, 37 women with subtype D, and 6 women with subtype C) for analysis, recognizing the limitation that different resistance assays were used for genotyping in the two trials. In this analysis, NVP-resistance mutations were detected in 10/36 (28%) of women with subtype C, which was similar to the rate of NVP-resistance seen in subtype D (11/37 = 30%) and higher than that seen in subtype A (6/48 = 13%)[23].

The HIVNET 012 NVP prophylaxis regimen is being implemented in countries around the world. If results from these exploratory studies are confirmed, they would suggest that the rate of NVP-resistance may vary among women receiving this regimen, depending on which subtypes are prevalent in a particular geographical region.

Clinical relevance of NVP-resistance following single dose NVP prophylaxis

The studies described above demonstrate that NVP-resistant HIV-1 can emerge in women and infants following single dose NVP prophylaxis. Emergence of NVP-resistant HIV-1 in this setting is not unexpected, and most likely reflects selection of pre-existing variants with NVP-resistance mutations. Previous studies suggest that HIV-1 variants with single drug resistance mutations are likely to exist at low levels in all HIV-infected patients prior to antiretroviral drug exposure[7,24]. NVP can rapidly inhibit replication of NVP sensitive viruses, permitting selection of NVP-resistant variants. The long half-life of NVP favors emergence of NVP-resistance. Most women in HIVNET 012 had relatively high viral loads and relatively low CD4 cell counts[14]. Since both of those factors were associated with emergence of NVP-resistance, women with less advanced HIV-1 disease may be less likely to develop NVP-resistance with this regimen. In settings where highly active antiretroviral therapy is available, initiation of fully suppressive antiretroviral therapy following NVP prophylaxis would most likely limit the emergence of NVP-resistant HIV-1.

It is not known whether emergence of NVP-resistant HIV-1 after single dose NVP prophylaxis will affect the efficacy of NVP prophylaxis in subsequent pregnancies. Available data suggests that NVP-resistance fades in women over time. Even though HIV-1 variants with NVP-resistance mutation may be maintained as minor variants, or as provirus in infected cells, the majority population of circulating HIV-1 12-24 months following NVP prophylaxis is likely to be NVP sensitive. This suggests that single dose NVP would remain effective for prophylaxis in subsequent pregnancies. Epidemiologic studies could be considered to examine the effectiveness of single dose NVP prophylaxis in future pregnancies. It is also not clear whether emergence of NVP-resistance following single dose NVP would limit use of NVP or other NNRTIs for subsequent treatment of HIV-1 infection. Clinical studies are needed to determine whether a sufficient reservoir of NVP-resistant virus is established in this setting to limit subsequent treatment options. In resource-poor countries where single dose NVP prophylaxis is most likely to be implemented, resources for treatment of HIV-1 infection are extremely limited. If antiretroviral treatment became more widely available in those countries, women and infants who received NVP prophylaxis could be offered treatment regi-

mens using other antiretroviral drugs. There is no evidence that transient detection of NVP-resistance after single dose NVP will affect the clinical progression of HIV-1 infection, or the risk of HIV-1 transmission HIV-1 to other adults. The studies described above found little evidence of mother-to-child transmission of NVP-resistant HIV-1 through breastfeeding. However, this requires further evaluation.

The relatively frequent emergence of NVP-resistance following single dose NVP prophylaxis emphasizes the need to evaluate the emergence of drug resistance among women receiving antiretroviral prophylaxis, particularly when regimens use antiretroviral drugs, such as NVP or lamivudine (3TC), in which a single mutation can confer resistance. The emergence of NVP-resistance must be balanced against the simplicity, efficacy, and cost-effectiveness of single dose NVP prophylaxis. The HIVNET 012 has been shown to significantly reduce HIV-1 MTCT in settings where other antiretroviral regimens are impractical, and where treatment options are limited. If rapidly implemented, this regimen has the potential to protect millions of infants from HIV-1 infection in the next decade.

Acknowledgements

This work was supported by 1) the HIV Network for Prevention Trials (HIVNET) and sponsored by the U.S. National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), Dept. of Health and Human Services (DHHS), through contract N01-AI-35173 with Family Health International, contract N01-AI-45200 with Fred Hutchinson Cancer Research Center, and subcontracts with JHU Makerere Univ (NOI-AI-35173-417); 2) the HIV Prevention Trials Network (HPTN) sponsored by the NIAID, National Institutes of Child Health and Human Development (NICH/HD), National Institute on Drug Abuse, National Institute of Mental Health, and Office of AIDS Research, of the NIH, DHHS (U01-AI-46745 and U01-AI-48054), and 3) the Adult AIDS Clinical Trials Groups (NIH, Division of AIDS, NIAID).

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