

# Nevirapine prophylaxis to prevent HIV-1 mother-to-child transmission: pharmacokinetic considerations in preterm infants

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## SUMMARY

Prophylaxis with zidovudine and 3 doses of nevirapine (NVP) is recommended for infants born to HIV-1 infected untreated mothers to prevent HIV-1 mother-to-child transmission. However little is known about NVP pharmacokinetics in neonates, mostly in preterm infants. We performed therapeutic monitoring of NVP plasma concentrations in a 32-week preterm HIV-1 exposed infant born to an infected untreated mother. With the recommended regimen, an intense NVP exposure was observed, with NVP plasma levels exceeding the target concentration by up to 40 times, suggesting that when a laboratory assessment of NVP plasma concentrations is available, it may be useful to monitor and optimize drug exposure.

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Mother-to-child transmission (MTCT) of HIV-1 can occur in utero, during labour and delivery, or postnatally through breast-feeding. Without intervention, the transmission rate is estimated to be 15-45%, but it dramatically decreases to about 1% when combination antiretroviral (ARV) regimens are given to a mother during the antenatal period (European Collaborative Study, 2005; Townsend *et al.*, 2008). Based on the Pediatric AIDS Clinical Trials Group (PACTG) 076, the 6-week neonatal zidovudine (ZDV) prophylaxis regimen is recommended for all HIV-exposed infants to further reduce perinatal transmission (Connor *et al.*, 1994). However for infants born to mothers who did not receive ARV during pregnancy, a prophylaxis with a two drug regimen of ZDV given for 6 weeks and 3 doses of nevirapine (NVP) in the first week of life is suggested by the Guidelines for the Prevention of HIV-1 Perinatal Transmission. The efficacy and safety of this two drug regimen have been recently demonstrated (Nielsen-Saines *et al.*, 2012; Panel on Treatment of HIV-Infected Pregnant Women and Prevention of Perinatal Transmission, 2012). Nevirapine is a potent non-nucleoside inhibitor of HIV-1 reverse transcriptase (inhibitory concentration 10 ng/ml) that rapidly reduces viral load, is well absorbed af-

ter oral administration and distributes widely throughout the body. Based on a substudy of the National Institute of Child Health and Human Development HIV-1 Prevention Trials Network 040/PATCG 1043 study, NVP in neonates is administered in 3 standardized doses by weight bands during the first week of life (12 mg if birth weight [BW] >2000 g, 8 mg if BW ≤2000 g) to maintain plasma NVP concentrations greater than the target of 100 ng/ml through the end of the second week of life (Mirochnick *et al.*, 2008). Data on NVP pharmacokinetics in term neonates showed a long half-life and high bioavailability of the drug. The time to reach the maximum concentration ( $t_{max}$ ) seems to be prolonged, meaning a lower absorption rate in newborns, while its clearance is in the range of the adult value and slower than in older infants (Mirochnick *et al.*, 2008). Little is known about NVP pharmacokinetics in preterm infants, but available data suggest highly variable NVP concentrations and  $t_{max}$  in this population, with a long half-life (Mugabo *et al.*, 2011). We describe therapeutic NVP monitoring in a preterm infant who received 6 weeks ZDV plus 3 doses of NVP.

A 32-week, 1728 g female infant was born to a 22-years-old mother by urgent cesarean section due to premature rupture of membranes and onset of labour. Apgar scores were 8 and 9 at 1 and 5 minutes respectively. Physical examination at birth was normal.

No serological maternal screening tests had been performed during pregnancy. Serological maternal tests performed at delivery showed negativity for HBsAg, anti-HCV and nontreponemal test for syphilis, but HIV-1 antibody reactivity. Intravenous (iv) ZDV was started in the infant 12 hours after birth pending the confirmatory test, at a

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dose of 1.5 mg/kg/dose twice daily. Breast-feeding was never started.

HIV-1 RNA PCR test revealed a maternal blood viral load of 883.502 copies/ml; CD4+ cell count was 588/mm<sup>3</sup>. Since the mother was unaware of her HIV status, she did not receive ARV treatment either during pregnancy or during labour and delivery.

An extended prophylactic regimen was started in the neonate: in addition to ZDV, 3 doses of NVP were administered in the first week of life (Mirochnick *et al.*, 2008). Oral NVP was administered in doses of 8 mg/dose: the first 38 hours after birth, the second 48 hours after the first dose (86h of life) and the third 96 hours after the second dose (182h of life). Routine therapeutic NVP plasma monitoring was performed, with blood samples of 1 mL taken 4 hours after each NVP dose and as close as possible to the next NVP dose.

The ZDV dose was maintained at 1.5 mg/kg/dose iv twice daily for 15 days; then it was increased to 2.3 mg/kg/dose iv twice daily for 20 days; from day 36<sup>th</sup> ZDV was administered orally at a dose of 3 mg/kg/dose twice daily until the end of the sixth week of life.

The infant never required respiratory support. Laboratory testing during the first week of life showed normal complete blood counts, hepatic, coagulative and renal function tests. Since the second week of life prophylaxis with folic acid 7.5 mg 3 times a week was administered. From the eighth day of life a reduction of the hemoglobin (Hb) value (from 15 g/dl at birth to 10.9 g/dl) was observed; on the 20<sup>th</sup> and 36<sup>th</sup> days of life severe reductions of Hb, reaching the values of 8.3 and 6.8 g/dl respectively required two red cell blood transfusions. Liver function tests always revealed normal hepatic function.

The infant was discharged on 38<sup>th</sup> day of life and was followed in the Neonatal Outpatient Unit of our Department. Virological and serological testing performed on the infant's blood sample until 18 months of life reflected prevention of MTCT, with persistent negative HIV-DNA PCR and HIV-RNA PCR and negative anti-HIV-1 antibodies from the 12<sup>th</sup> month of life.

For detection of anti-HIV-1 antibodies and HIV p24 antigen a fourth-generation automated anti-HIV enzyme immunoassay (EIA), (Architect HIV Ag/Ab Combo, Abbott Diagnostics) was used. CD4+ lymphocyte and free HIV virions (HIV RNA) were determined by flow cytometry (FACScan, Becton & Dickinson, Mountain View, CA, USA) using commercially available monoclonal antibodies and by Versant kPCR (Siemens Healthcare Diagnostic, Tarrytown, NY, USA) respectively. Determination of HIV-1 proviral DNA in blood mononuclear cells was performed

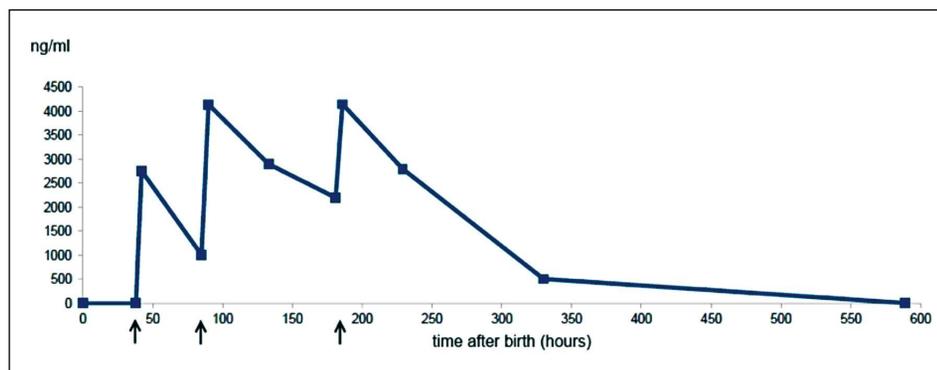
using SYBR Green real-time PCR (De Rossi *et al.*, 2010; Re *et al.*, 2010).

For NVP analysis blood samples of 1 mL were taken. Blood serum was isolated by centrifugation at 3500 rpm for 5 minutes, separated and stored at -20°C for subsequent analytical processing. Plasma NVP concentrations were measured by a specific Liquid Chromatography-Tandem Mass Spectrometry method. Analysis was performed by an i-Methods™ Test for Antiretrovirals (AB Sciex, USA), which provides specific, sensitive (LOD=0.1 ng/ml) and accurate measurement (BIAS<5% at all concentration level tested), and further in-house validated on an API4000 instrument (AB Sciex, USA) and controlled with reference materials and internal standards (Anti-HIV Drugs Calibration Standards, Chromsystems, Germany). Concentration over time curve fitting was performed by PKsolver freeware running in Microsoft Excel (Zhang *et al.*, 2010).

Little is known about NVP pharmacokinetics in preterm infants. A study by Mugabo *et al.* (2011) analyzed NVP pharmacokinetics in preterm infants exposed to a single dose (sd) of NVP born to mothers who either received or did not receive NVP during labour. In this study the NVP dosing was 2 mg/kg for infants with BW <2000 g and 6 mg total if BW >2000g. With this regimen the NVP concentration on day 8 exceeded the target of 100 ng/ml in 78% of infants whose mothers had received sd NVP and 70% of infants whose mothers had not received it (Mugabo *et al.*, 2011).

Given the lack of data on NVP pharmacokinetics in premature infants receiving prophylaxis with the regimen of ZDV and 3 NVP doses in the first weeks of life, we performed therapeutic monitoring of NVP concentration to verify the adequacy of therapy. Since we used 3 NVP doses of 8 mg each, the treatment reflects a 4.6 mg/kg dose.

Analyzing the NVP concentration to time curve (Fig. 1), we found an intense exposure to NVP up to 14<sup>th</sup> day of life. NVP C<sub>max</sub> was reached 4 hours after the third dose reaching a value of 4146 ng/mL (t<sub>max</sub> 186h), which is 40 times greater the 100 ng/ml target for drug efficacy. This value is similar to that reported in the previous study on preterm neonates exposed to sd NVP (Mugabo *et al.*, 2011), where the C<sub>max</sub> range in infants born to mothers who received sd NVP during labour was 350-3832 ng/ml (t<sub>max</sub> 25h50, range 9h40-83h45). Recently another study analyzed NVP concentrations in preterm infants following the World Health Organization recommended regimen of 4 weeks 2 mg/kg/day for HIV-exposed infants <2000 g. Their results also showed elevated plasma concentrations without significant toxicity (de Waal *et al.*, 2014).



**Figure 1** - Graph obtained with PK Solver 2.0 for a non-compartmental analysis of plasma data.

Drug exposure, measured by the AUC parameter (AUC = 824.000 ng/ml\*h) was, as expected, slightly higher than the median value of AUC previously reported and reveals an intense exposure to the drug, likely related to decreased NVP elimination. The median increase in NVP blood concentration after each dose was 567 ng/ml per mg/kg dose (range 423-680 ng/ml per mg/kg), with the minimum increase recorded after the third dose, which probably reflects auto-induction of NVP metabolism or maturation of hepatic function. The intense NVP exposure may be related to several age-related factors, such as gastric acid secretion, gastric emptying time, intestinal transit time, intestinal motility, cytochrome activity, which changed during the first weeks of life. All these factors could influence the different drug absorption in premature neonates. It is of concern that the intense NVP exposure could increase the risk of developing HIV genotypic resistance to NVP for patients who go on to become infected despite prophylaxis. Furthermore, the drug could increase the risk of adverse effects. In our patient, the commonest short-term toxicities related to NVP were not observed, but she showed a severe anaemia, possibly as a consequence of therapy with both NVP and ZDV in an infant at high risk of anaemia, because of preterm birth.

Unfortunately, since our goal was to verify the adequacy of therapy, we did not perform a more intensive sampling and this means that we cannot ascertain if we missed a peak NVP concentration, with a possible underestimation of the AUC and consequently of the true higher concentration experienced by the infant.

This report highlights the opportunity to optimize the posology of NVP prophylaxis in preterm infants. We found NVP plasma levels exceeding the target concentration by up to 40 times, with high values maintained until the 14<sup>th</sup> day of life. The standardized dosing of NVP in preterm infants has the advantage of minimizing the complexity of the regimen to make it more practical in resource-sparse settings. However, when a laboratory assessment of NVP plasma concentrations can be obtained, adapting the dose on the basis of the individual response may be a suitable option to prevent the emergence of drug-resistant isolates and avoid potential toxicities, and to maintain an acceptable NVP concentration in preterm infants. Further stud-

ies involving a larger population could better clarify NVP pharmacokinetics and its optimum dosing in preterm infants.

## Abbreviations

ARV- antiretroviral therapy; BW - birth weight; Hb - hemoglobin; IV - intravenous; MTCT - Mother-to-child transmission; NVP - nevirapine; sd - single dose; ZDV - zidovudine.

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