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Prevalence of resistance to nevirapine in mothers and children after single-dose exposure to prevent vertical transmission of HIV-1: a meta-analysis†

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Background Single-dose nevirapine (NVP) is the main option for the prevention of mother-to-child transmission (PMTCT) of HIV-1 in countries with limited resources. However, the use of single-dose NVP results in HIV-1 viral resistance which could compromise the success of subsequent treatment of mother and child with antiretroviral combinations that include non-nucleosidic-reverse-transcriptase inhibitors. This systematic review and meta-analysis of summarized data aimed to estimate the proportion of mothers and children with NVP resistance mutations detected in plasma samples 4–8 weeks postpartum after single-dose NVP use for PMTCT.

Methods Systematic search of electronic databases (MEDLINE, PASCAL) and conference proceedings (1997 to February 2006). Inclusion of all studies, without design, place or language restrictions, meeting the following criteria: use of single-dose NVP; viral genotyping performed with standard sequence analyses, between 4 and 8 weeks postpartum, in plasma samples; available public report; report of mothers' median baseline plasma HIV-1 RNA levels. Data extraction by two independent reviewers using a standardized form created for this purpose.

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Logistic random effect models to obtain pooled estimates. Univariable and multivariable meta-regression to explore sources of heterogeneity.

Results The pooled estimate of NVP resistance prevalence was 35.7% [95% confidence interval (CI) 23.0–50.6] in women in 10 study arms using single-dose NVP ± other antepartum antiretrovirals and 4.5% (CI 2.1–9.4) in three study arms providing also postpartum antiretrovirals (adjusted odds ratio 0.08; CI 0.04–0.16). The corresponding estimates in children were 52.6% (CI 37.7–67.0) in seven study arms using single-dose NVP only and 16.5% (CI 8.9–28.3) in eight study arms combining single-dose NVP with other antiretrovirals.

Conclusions Single-dose NVP is widely used for PMTCT in resource-poor settings, but the burden of viral resistance is high in both women and children. It is substantially lower in studies providing additional postpartum antiretrovirals. The clinical implications of these findings should be further investigated.

Keywords PMTCT, HIV-1, nevirapine resistance, meta-analysis, systematic review

Introduction

In 2006, an estimated 2.3 million children under 15 years were living with HIV/AIDS worldwide and at least 380 000 died of AIDS.[1] In the same year, almost 530 000 infants became newly infected with HIV, 90% of them through mother-to-child transmission (MTCT) before, during or after delivery.[1–3] Transmission around the time of delivery accounts for nearly half of overall MTCT in breastfeeding populations,[2,3] and this peri-partum period has become the major focus of prevention of MTCT (PMTCT) strategies, especially with antiretroviral drugs (ARVs). Single-dose nevirapine (NVP) given to the delivering woman and to the neonate within 72 h of birth has been proven to be a safe and effective intervention to prevent MTCT.[4,5] The regimen is cheap and relatively easy to administer. Since 2000, single-dose NVP has been endorsed by the World Health Organization (WHO) as one of several recommended ARV regimens for PMTCT in resource-limited settings.[6]

Single-dose NVP induces the selection of HIV-1 resistant mutants in mothers and infants. However, the extent of this problem is poorly quantified with estimated prevalence ranging from 1 to 69% for women and from 0 to 87% for neonates.[7–23] Although it has been suggested that NVP-induced resistance after exposure for PMTCT may impair virological response to the subsequently used non-nucleosidic-reverse-transcriptase inhibitors (NNRTIs) in mothers and infants,[24,25] this was not confirmed in recent studies[26–28]and the clinical implications remain unclear.[20,28,29]

Little is known about factors associated with the development of NVP viral resistance, but it is likely that high HIV-1 RNA levels in plasma, low CD4 cell counts, viral subtype and NVP plasma concentration play a role.[7,30] Recent preliminary results from a randomized trial in South Africa (Treatment Options Preservations Study or TOPS) suggest that the use of a short (4 or 7 days) post-partum regimen of zidovudine (ZDV) + lamivudine (3TC) given to the mother after single-dose NVP intake substantially reduces the rate of NVP resistance,[21] and this was confirmed in an observational study in Côte d'Ivoire.[8]

We performed a systematic review and a meta-analysis of available data to estimate the prevalence of NVP viral resistance mutations in mothers and HIV-1-infected children and explore sources of between-study heterogeneity, including additional ARV interventions given post-partum.

Methods

Search strategy

MEDLINE and PASCAL were searched for published articles between 1997 and February 2006 using a combination of the terms 'Resistance AND [(nevirapine OR NVP) OR NNRTI]'. Searches were complemented by perusing references of retrieved articles. Studies presented at conferences (US Conference on Retroviruses and Opportunistic Infections, International AIDS Conference, International HIV Drug Resistance Workshop, Conference on HIV Pathogenesis and Treatment; 2000–06) were also searched using NLM Gateway®, conference websites or conference proceedings.

Inclusion criteria

All studies, without design, place or language restrictions, were considered if they met the following four selection criteria: (i) a single-dose NVP had been given to HIV-1 infected women during labour, (ii) viral genotypic resistance assay had been performed with sequence analyses (detection limit ~20–30%), between 4 and 8 weeks post-partum, in mothers and children's plasma samples, (iii) an abstract, an article or an oral or poster presentation was available and (iv) for the meta-analysis on mothers' findings only, median baseline plasma HIV-1 RNA level was reported.

When a study had two or more intervention arms, only those where women had received single-dose NVP were included in the meta-analysis. When multiple communications were available for the same study, we included the most recent one and/or the one with the largest number of observations. The homogeneity relative to the genotypic assessment methods used in the included studies was checked by the virologists. Studies using sensitive drug resistance assays (detection limit <1%) in plasma sample were retained for a complementary analysis to estimate the proportion of patients with NVP viral resistance mutations using these methods.

Data extraction

Two independent observers (EA, DKE) independently assessed the eligibility of studies using a standardized form developed for this purpose. Disagreements between reviewers were resolved through discussion. Data extraction was done by the same reviewers using a standardized data extraction form created for this study and with help from others when needed (RT, BM, PVDP, CR). The following information was obtained from each study: first author's name, journal and year of publication/presentation and details on the principal study in case the resistance study was a substudy; patient inclusion and exclusion criteria in the primary study and in the resistance study; place of study; ARV intervention(s); methods used for resistance testing; median baseline plasma HIV-1 RNA viral load; and for all assessments, when available, timing, number of available samples, number of successful analyses, number of study participants with NVP resistance mutations. We asked authors of primary studies to check the accuracy of extracted data and supply additional data if required.

Outcome measures

The outcomes of interest were the proportions of mothers and children with NVP resistance viral mutations in codons of the viral reverse transcriptase gene known to confer resistance to NVP (100, 103, 106, 108, 181, 188, 190)[31] detected in plasma samples collected between 4 and 8 weeks post-partum.

Data synthesis and exploration of heterogeneity

The proportion of study participants with NVP resistance mutations from each selected study was graphically displayed with the 95% confidence interval (CI) in a forest plot. The overall prevalence of NVP resistance mutations and its 95% CI was estimated with a random effect logistic model (PROC NLMIXED in SAS software version 8.2 USA).[32,33] The number of degrees of freedom was calculated as the number of study arms minus the number of parameters estimated in the model.

Between-study heterogeneity was assessed using the likelihood ratio test of the random effect component which had a 50 : 50 mixture distributions of X^2_{q+1} and X^2_q, asymptotically (where q was the number of parameter estimates under the null hypothesis). Univariable and multivariable meta-regressions were conducted to quantify potential sources of heterogeneity[34,35] such as study and report characteristics. Study characteristics included:

(i) Administration of ante/intrapartum ARV regimen in addition to single-dose NVP
(ii) Administration of post-partum ARV regimen in addition to single-dose NVP
(iii) Study location: in Southern Africa vs other regions
(iv) Median baseline plasma HIV-1 RNA viral load, dichotomized at the median value
(v) Time of resistance assessment: at 4 weeks vs 6–8 weeks post-partum
(vi) Sample size, dichotomized at 30 study participants
(vii) Reported pre-NVP exposure resistance assessment
(viii) Exhaustivity of the sample with resistance assessment compared with the sample enrolled in the main study.

Report characteristics included:

(i) Publication type
(ii) Status of the reported results: final or preliminary.

Variables with P-value <0.20 in univariable models were examined in multivariable models using a forward stepwise procedure. When between-study variance failed to be calculated, no further independent variable was added in the random effect models and fixed effects models were used. All P-values were two-tailed.

Results

Prevalence of resistance in mothers

Among 74 reports identified from 20 investigators, 64 were excluded because they were either duplicates or met exclusion criteria (Figure 1). The investigators were contacted and eight responded. Seven reports could thus be updated, one still failing to meet every inclusion criterion. Answers from the other investigators would have allowed the re-evaluation for eligibility of four additional studies.

Ten reports were thus included in the final analysis, relating to nine studies reported between 2000 and 2006 and corresponding to 13 study arms and 1173 women overall. The arms of the studies included in the meta-analysis were described in Table 1.

Of the nine included studies, one (the TOPS trial)[21] had three post-partum intervention arms, two [the ANRS (Agence Nationale de Recherche sur le Sida et les hépatites virales)] 1201 DITRAME (DIminution de la TRAnsmission Mère-Enfant) Plus cohort[7,8] and the South African PMTCT cohort)[20] had two intervention arms (Table 1). Four studies took place in Southern Africa (South Africa, Zimbabwe and Malawi), and two in Eastern Africa (Uganda). Published articles were available for six studies, as well as an unpublished report for one of them, poster/slides for two studies and an abstract for one. Results were reported as final for six studies. Genotypic resistance assessment prior to single-dose NVP use in women with NVP resistance at 4–8 weeks post-partum or in a subset had been described for six studies. The median of the reported median baseline plasma HIV-1 RNA viral loads was 4.39 $\log_{10}$ copies/ml [interquartile range (IQR): 4.00–4.48].

In univariable meta-regression (Table 2), both the use of ante- and post-partum ARV regimens were associated with a lower NVP resistance prevalence, as was a higher median baseline plasma HIV-1 RNA viral load. The univariable inverse relation between baseline HIV-1 RNA viral load and occurrence of NVP resistance did not hold when this variable was adjusted for post-partum ARV intervention. Development of NVP resistance mutations was more frequent in studies in Southern Africa. In multivariable meta-regression (Table 2), no more than two independent variables could be added in the random effect model. Post-partum ARV regimen, study location and baseline median viral load remained strongly associated with the detection of NVP resistance mutations.

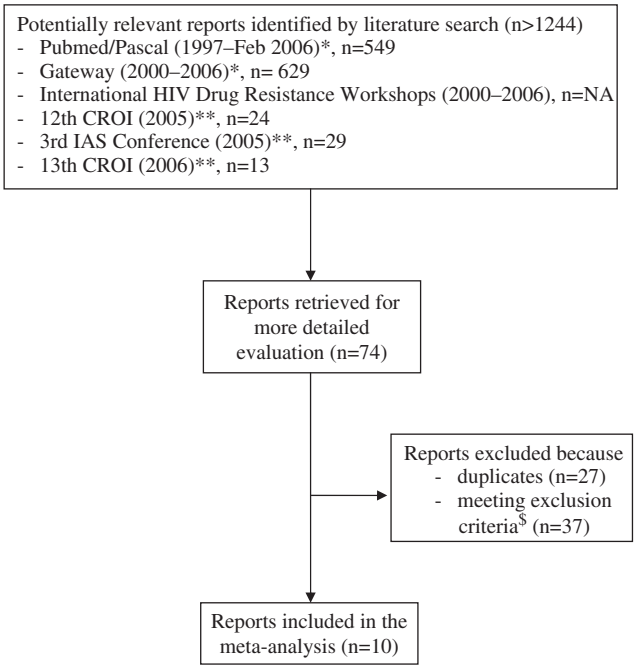

Figure 1 Flow chart of studies included in the meta-analysis on prevalence of viral resistance to nevirapine in mothers after single-dose exposure to prevent vertical transmission of HIV-1. *Terms of search: ''Resistance AND ((nevirapine OR NVP) OR NNRTI)''. NNRTI = non nucleosidic reverse transcriptase inhibitors. **Terms of search: ''nevirapine'' and ''NVP''. CROI = Conferences on Retroviruses and Opportunistic Infection. IAS = International AIDS Society. NA = not available. $See definition in the text

Using univariable random effect models with the post-partum ARV regimen variable (Figure 2), the pooled estimate of NVP resistance prevalence in the 10 study arms using single-dose NVP ± other ante/intra-partum ARVs was 35.7% ($n = 950$, CI: 23.0–50.6), and 4.5% ($n = 223$, CI: 2.1–9.4) in three study arms providing also post-partum ARVs.

Complementary analysis from results using sensitive drug resistance assays in mothers

Five studies using sensitive drug resistance assays were identified (Table 3). We excluded from the analysis the women who received other ARV in addition to single-dose NVP (arms 2 and 3 of the TOPS trial). Using a random effect model with no explanatory variable, the pooled estimate of NVP resistance prevalence was 62.4% ($n = 423$; CI: 41.7–79.5).

Prevalence of resistance in offspring

Among 35 relevant reports, 24 were excluded because they were either duplicates or met exclusion criteria (Figure 3), giving 11 reports for inclusion in the final analysis, relating to 10 studies reported between 2000 and 2006, corresponding to 15 study arms and 339 children overall. Five of these studies were also included in the meta-analysis of NVP resistance in mothers. The arms of the studies included in the meta-analysis were described in Table 4.

Of the 15 study arms, four included both a maternal ante/intrapartum ARV intervention and an infant ARV prophylaxis with ZDV added to single-dose NVP.[7,8,13,22] In one study arm, a second post-partum dose of NVP was provided[23] and in two other arms, both maternal postpartum and neonatal ZDV + 3TC interventions were given[21] (TOPS arms 2 and 3). Another arm provided ante/intrapartum and postpartum ZDV + 3TC plus infant ZDV prophylaxis[8] and another one, infant ZDV prophylaxis only.[13] Six studies took place in Southern Africa (South Africa and Malawi). All studies performed the genotypic assessment at 6–8 weeks post-natal. Median sample size was 23 (IQR: 10–29). An article was available for three studies, as well as an unpublished report for one of them, conference presentations (slides or posters) for four studies and abstracts for three.

In univariable meta-regression (Table 5), antepartum, post-partum and post-natal ZDV or ZDV + 3TC interventions were associated with lower NVP resistance prevalence in children. Location in Southern Africa was associated with higher NVP resistance. In multivariable analysis (Table 5), antepartum and post-partum ARV interventions remained strongly associated with lower NVP resistance prevalence in children, but not neonatal ARVs and geographical location.

Using a univariable random effect model, the pooled estimates of NVP resistance prevalence were 52.6% ($n = 201$; CI 37.7–67.0) in the group of seven study arms using NVP only and 16.5% ($n = 138$; CI 8.9–28.3) in the group of eight study arms combining NVP with ZDV or ZDV + 3TC interventions (Figure 4).

Discussion

We estimated that about one-third of women and more than half of children who became HIV-1-infected despite PMTCT, developed NVP resistance mutations after single-dose NVP intake, but this rate was reduced to about 4% and 16%, respectively, if additional short-course ARV regimens were given, post-partum ZDV and 3TC in particular. These pooled estimates should be interpreted with caution as the heterogeneity among the intervention groups remained important due to other factors assessed in the multivariable analyses.

A strength of our study is our effort to assess potential sources of between-study heterogeneity. Although we included only reported studies, our systematic review had a comprehensive coverage, thus limiting publication bias. Indeed, we believe that all NVP resistance studies which have been completed or are in progress, have been reported, whatever their results, at least as a conference communication.

The outcome of interest was standardized between studies, and we restricted our analysis to results of plasma samples acknowledging that NVP resistance mutations may differ between compartments (peripheral blood mononuclear cells or breast milk).[7,17]

The genotypic resistance assessment methods, all sequence analyses, were reasonably equivalent between studies included in the meta-analyses. We did not include studies using the more sensitive recent techniques[36–40] in the main analysis, partly because the clinical implications of

Table 1 Characteristics of resistance studies included in the meta-analysis on prevalence of resistance to nevirapine in mothers after single-dose nevirapine to prevent vertical transmission of HIV-1

References	Type	Place	Study name	Design	Arms with intervention of interest		Reported data	Exhaustivity[a]	Method of assessment	Pre-NVP exposure genotypic assessment	Time of genotypic assessment	Median baseline viral load	Size	% NVPR
					Mothers	Infants								
Martinson *et al.*[20]	Slides	South Africa	PMTCT program	OS	sdNVP previously exposed naive	NI	Preliminary results	NI	ViroSeq HIV-1 Genotyping system (Applied Biosystems)	In all women in the resistance study	6 weeks	3.7 4	51 92	45.1 55.4
McIntyre *et al.*[21]	Slides	South Africa	TOPS	RCT	Arm 1: Intrapartum sdNVP Arm2: Intrapartum sdNVP + 4 days CBV Arm3: Intrapartum sdNVP + 7 days CBV	Arm 1: sdNVP Arm2: sdNVP + 4 days CBV Arm3: sdNVP + 7 days CBV	Preliminary results	Exhaustive sample	Sequencing analyses	In all women of the trial	6 weeks	4.37 4.39 4.54	68 67 68	60.3 11.9 10.3
Eshleman *et al.*[12]	Article	Malawi	NVAZ	RCT	Intrapartum sdNVP		Final results	NI	ViroSeq HIV-1 Genotyping system (Applied Biosystems)	In a subset of women	6–8 weeks	4.78	65	69.2
Lee *et al.*[17]	Article	Zimbabwe	HPTN 023	RCT	Intrapartum sdNVP	NI	Final results	NI	TruGen HIV-1 kit (Visible Genetics)	NI	8 weeks	4.4	32	34.4
Chaix *et al.*[7,8]	Article and unpublished technical report	Côte d'Ivoire	ANRS 1201 DITRAME Plus 1.0 and 1.1	OS	1.0: Antenatal ZDV + intrapartum ZDV + sdNVP 1.1: Antenatal ZDV + 3TC + intrapartum ZDV + 3TC + sdNVP + postpartum 3 days ZDV + 3TC	sdNVP + 1 week ZDV sdNVP + 1 week ZDV	Final results	Selected sample	Sequencing reverse transcriptase gene + Sequence Navigator Software (Applied Biosystems PE)	In all women with NVP viral resistance detected in further assessment	4 weeks	4.64(1) 4.48(1)	63 88	33.3 1.14
Eshleman *et al.*[11]	Article	Uganda	HIVNET 012	RCT	Intrapartum sdNVP	sdNVP	Final results	Exhaustive sample	ViroSeq HIV-1 Genotyping system (Applied Biosystems)	In a subset of women	6 weeks	4.4	279	25.1
Jackson *et al.*[16]	Article	Uganda	HIVNET 006	OS	Intrapartum sdNVP	+/− sdNVP	Final results	Exhaustive sample	ViroSeq HIV-1 Genotyping system (Applied Biosystems)	In all women with NVP viral resistance detected subsequently	6 weeks	4.36	15	20.0
Chalermchok-charoenkit *et al.*[9]	Abstract	Thailand	Thailand CDC	OS	Antenatal ZDV + Intrapartum ZDV + sdNVP	sdNVP + 4 weeks ZDV	NI	Exhaustive sample	TruGen HIV-1 kit (Visible Genetics)	NI	4 weeks	3.51(2)	190	17.4
Cunningham *et al.*[10]	Article	Multicountry (USA, Europe, Brasil)	PACTG 316	RCT	Non study ARV + intrapartum ZDV +/− sdNVP	sdNVP or placebo	Final results	Selected sample	TruGen HIV-1 kit (Visible Genetics) + Sequence Navigator Software (Applied Biosystems PE)	In women with NVP viral resistance detected in further assessment	6 weeks	3.48(2)	95	14.7

RCT: randomised controlled trial; OS: Observational study; NI: no information; NVPR: NVP resistance; ZDV: Zidovudine; sdNVP: single-dose Nevirapine; 3TC: Lamivudine; CBV: Combivir® (ZDV + 3TC); For study arms with antepartum ARV intervention: (1)plasma viral load performed at inclusion (2)plasma viral load performed at delivery; PMTCT: Prevention of Mother-To-Child-Transmission; TOPS: Treatment Options Preservations Study; NVAZ:Nevirapine-AZT (zidovudine); HPTN: HIV Prevention Trials Network; ANRS: Agence Nationale de Recherches sur le Sida et les hépatites virales; DITRAME: DIminution de la TRAnsmission Mere-Enfant; HIVNET: HIV Network for Prevention Trial; CDC: Center for Diseases Control; PACTG: Pediatric AIDS Clinical Truials Group Protocol.

[a]Exhaustivity of the sample size with genotypic assessment compared with the number of participants included in the main study.

Table 2 Univariable and mutivariable meta-regression on prevalence of nevirapine (NVP) viral resistance in women at 4–8 weeks postpartum after single-dose exposure to prevent vertical transmission of HIV-1

Variables	Univariable				Multivariable				Multivariable	
	Random effect models				Random effect models				Fixed effect model	
	OR [CI][a]	*P*-value	OR [CI][a]	*P*-value	OR [CI][a]	*P*-value	OR [CI][a]	*P*-value	OR [CI][a]	*P*-value
Postpartum ARV intervention Yes vs No (Ref.)	0.07 [0.03–0.15]	$<10^{-4}$	–	–	0.08 [0.04–0.16]	$<10^{-4}$	0.04 [0.01–0.14]	$<10^{-3}$	0.07 [0.03–0.13]	$<10^{-4}$
Ante/Intrapartum ARV intervention Yes vs No (Ref.)	0.29 [0.12–0.75]	0.016	0.6 [0.20–1.72]	0.3	–	–	–	–	–	–
Study location Southern Africa vs other regions (Ref.)	3.81 [1.73–8.40]	0.004	2.7 [0.97–7.46]	0.06	4.69 [2.58–8.51]	$<10^{-3}$	–	–	5.05 [3.57–7.14]	$<10^{-4}$
Median baseline median plasma viral load >4.39 $\log_{10}$ copies/ml vs $\leqslant$4.39 (Ref.)	0.11 [0.05–0.23]	$<10^{-4}$	–	–	–	–	1.93 [0.61–6.16]	0.23	1.53 [1.08–2.17]	0.022
Time of resistance assessment 4 weeks vs 6–8 weeks postpartum (Ref.)	0.37 [0.10–1.32]	0.111	–	–	–	–	–	–	–	–
Exhaustivity[b] Yes vs No (Ref)	1.81 [0.54–6.00]	0.298	–	–	–	–	–	–	–	–
Status of the results Preliminary or no information vs final (Ref.)	1.09 [0.44–2.69]	0.838	–	–	–	–	–	–	–	–
Sample size <30 vs $\geqslant$30 (Ref.)	0.56 [0.05–6.14]	0.599	–	–	–	–	–	–	–	–
Pre-NVP exposure resistance assessment Yes vs no information (Ref.)	0.74 [0.16–3.35]	0.669	–	–	–	–	–	–	–	–
Publication type Articles vs other types (Ref.)	0.10 [0.02–0.41]	0.004	–	–	–	–	–	–	–	–
Between-study variance	–	–	–	$<10^{-3}$	–	0.314	–	$<10^{-4}$	–	–

[a]Odds Ratio [Limits of 95% confidence interval]. ARV: antiretroviral.

[b]Exhaustivity of the sample size with resistance assessment compared with the number of participants included in the main study.

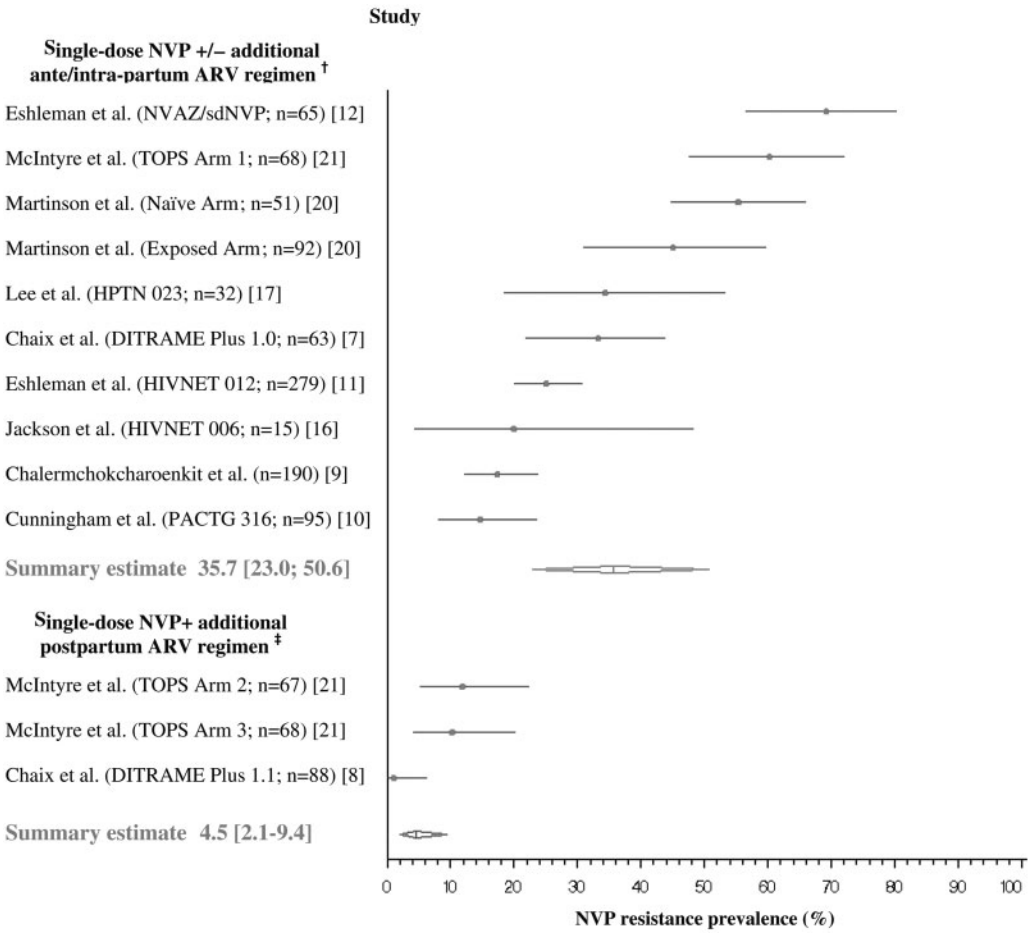

Figure 2 Forest plot of prevalence of viral resistance to nevirapine (NVP) in mothers at 4–8 weeks postpartum after single-dose exposure, grouped according to whether mothers received or not additional postpartum antiretroviral (ARV) therapy (univariable random effect model). †Zidovudine or ARV combination. ‡Zidovudine and lamivudine. TOPS: Treatment Options Preservations Study. NVAZ:Nevirapine-AZT (zidovudine). HPTN: HIV Prevention Trials Network. DITRAME: DIminution de la TRAnsmission Mere-Enfant. HIVNET: HIV Network for Prevention Trial. PACTG: Pediatric AIDS Clinical Trials Group Protocol

their results were unclear and partly because we did not want to introduce further heterogeneity and data overlap. However, complementary analysis of only studies using these more sensitive NVP-resistance assays showed a prevalence of resistance of 62%, and our results should thus be considered a minimum estimate of prevalence of NVP resistance. Finally, it has been previously reported that NVP resistance mutations could fade over time.[14] The NVP resistance rates reported in this meta-analysis were all observed within a homogeneous short post-partum treatment period that ranged between 4 and 8 weeks. This increases the validity of our pooled estimates.

Our study has several limitations. First, reporting bias could have affected our findings. Because of lack of information, we were unable to adjust for treatment characteristics such as the percentage of women who received a second dose of NVP, after false or prolonged labour, or HIV-1 subtypes which have previously been identified to be associated with NVP resistance.[13] However, we could adjust for study location, thus taking indirectly into account HIV-1 subtypes which are geographically distributed. In the meta-analysis in children, we could not evaluate the effect of duration of post-natal prophylaxis or that of regimen (ZDV only vs ZDV + 3TC) due to the very limited number of reported studies. Some data were only available from abstracts or internal reports, although these were not yet peer-reviewed. However, most of them had already been presented at several occasions in international conferences, which provides some guarantee of quality. Secondly, although the indirect comparisons we have conducted could be subject to greater bias than a randomized design (especially selection bias), in the absence of such 'ideal' studies, the model we used was appropriately adapted to the complex nature of the data.[41]

Exposure to antepartum ARVs was not associated with resistance risk in mothers in multivariable analysis. However, this may be due to the fact that it was administrated in one of the two studies providing also post-partum ARVs. Similarly, ZDV syrup was routinely given to neonates in studies where mothers were offered antenatal ARV intervention, before single-dose NVP and it was not possible to distinguish the effect of maternal and neonatal ZDV on the risk of NVP resistance in infants. Considering the different resistance profiles described

Table 3 Characteristics of resistance studies using sensitive drug-resistance assays on prevalence of resistance to nevirapine in mothers after single-dose nevirapine to prevent vertical transmission of HIV-1

References	Type	Place	Study	Arms with intervention of interest	Exhaustivity[a]	Method of assessment	Limit of detection	Sub-type	Time of genotypic assessment	Median baseline viral load	Size	% NVPR
Johnson *et al.*[35]	Article	South Africa	PMTCT program	Intrapartum sdNVP	NI	Sensitive real-time PCR for K103N and Y181C	0.2%	C	6–36 weeks	4.36	50	62.0
Palmer *et al.*[39]	Abstract	South Africa	TOPS	Arm 1: Intrapartum sdNVP Arm2/3: Intrapartum sdNVP+ 4/7 days CBV	Subset	Allele-specific real-time-PCR for 103N and 181C	0.1% 0.1%	C C	6 weeks 6 weeks	NI NI	10 22	75.0 27.3
Loubser *et al.*[37]	Abstract	South Africa	PMTCT program	Instrapartum sdNVP	NI	Sensitive real-time PCR for K103N	0.2%	C	6 weeks	NI	18	88.9
Flys *et al.*[38]	Article	Uganda Malawi	HIVNET 012 NVAZ	Intrapartum sdNVP Intrapartum sdNVP	Subset Subset	LigAmp for K103N LigAmp for K103N	0.5% 0.5%	A D C	6-8 weeks 6-8 weeks 6-8 weeks	NI NI NI	144 94 63	41.7 54.2 69.8
Lehman *et al.*[36]	Abstract	Kenya	PMTCT program	Intrapartum sdNVP	NI	Allele-specific RT-PCR for K103N	NI	NI	4 weeks	NI	30	40.0

NI: no information; PMTCT: prevention of mother-to-child transmission; NVPR: NVP resistance; sdNVP: single-dose Nevirapine; CBV: Combivir® (Zidovudine+lamivudine); TOPS: Treatment Options Preservations Study; NVAZ:Nevirapine-AZT (zidovudine); HIVNET: HIV Network for Prevention Trial.
[a]Exhaustivity of the sample size with genotypic assessment compared with the number of participants included in the main study.

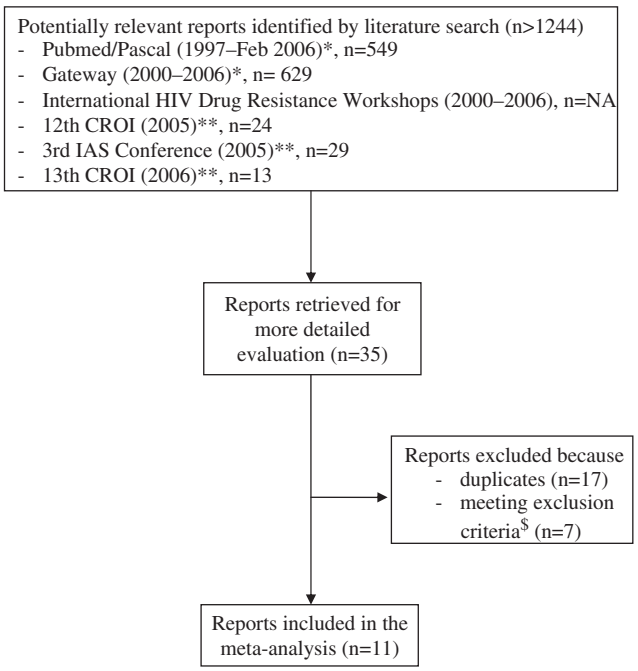

Figure 3 Flow chart of studies included in the meta-analysis on prevalence of viral resistance to nevirapine in children after single-dose exposure to prevent vertical transmission of HIV-1. *Terms of search: ''Resistance AND ((nevirapine OR NVP) OR NNRTI)''. NNRTI = non nucleosidic reverse transcriptase inhibitors. **Terms of search: ''nevirapine'' and ''NVP''. CROI = Conferences on Retroviruses and Opportunistic Infection. IAS = International AIDS Society. NA = not available. $See definition in the text

in mothers (K103N) and children (Y181C) in most studies included in this meta-analysis,[8,14,15,18,19,23] the acquisition of resistant virus may be more likely to be due to a sub-optimal neonatal prophylaxis than to the transmission of such virus by the mother. In one study, the K103N was the most common mutation observed in children[22] and the authors suggested this could be in relation with the pressure of ZDV prophylaxis on the CRF01_AE subtype.

In the meta-analysis on NVP resistance in mothers, study location in Southern Africa was independently associated with a higher NVP resistance prevalence, which could be due, in part, to the high prevalence of viral subtype C.[12,42] In Côte d'Ivoire, where the prevalence of NVP resistance prevalence was lowest and where women received additional antepartum and post-partum ARVs, the main subtype is CRF02-AG. However, other reports have shown resistance frequencies similar to those observed in other countries where other variants predominate: 33.3% in a group of women receiving additional ante/intra-partum ARVs[7] and 20.7% in a group of women receiving single-dose NVP only[43] (publication not included in our analysis due to the lack of availability of baseline plasma RNA viral load).

Allowing for study location and post-partum intervention, we also confirmed that the prevalence of NVP resistance increased with higher baseline HIV-1 RNA viral load.[6] Although this finding should be interpreted with caution due to the risk of aggregation bias,[35] this result raises a potential case management problem as these women are likely to become

Table 4 Characteristics of resistance studies included in the meta-analysis on prevalence of resistance to nevirapine in children after single-dose nevirapine to prevent vertical transmission of HIV-1

References	Type	Place	Study	Design	Arms with intervention of interest: Mothers	Arms with intervention of interest: Infants	Reported data	Exhaustivity[a]	Method of assessment	Time of genotypic assessment	Size	% NVPR
McIntyre *et al.*[21]	Slides	South Africa	TOPS	RCT	Arm 1: Intrapartum sdNVP Arm2: Intrapartum sdNVP + 4 days CBV Arm3: Intrapartum sdNVP + 7 days CBV	Arm 1: sdNVP Arm2: sdNVP + 4 days CBV Arm3: sdNVP + 7 days CBV	Preliminary results	Exhaustive sample	Sequence analysis	6 weeks	9 8 7	77.8 12.5 0
Sullivan *et al*[23]	Abstract	South Africa	SAINT	RCT	Intrapartum sdNVP + Postpartum sdNVP	sdNVP	NI	NI	NI	4-6 weeks	40	52.5
Eshleman *et al.*[13]	Article	Malawi	NVAZ	RCT	Intrapartum sdNVP	Arm 1: sdNVP Arm 2: sdNVP + 7 days ZDV	Final results	NI	ViroSeq HIV-1 Genotyping system (Applied Biosystems®)	6–8 weeks	20 21	87.0 57.1
Loubser *et al.*[18]	Poster	South Africa	PMTCT program	OS	sdNVP	sdNVP	NI	NI	RT-PCR and DNA sequencing	6 weeks	25	36.0
Gordon *et al.*[15]	Abstract	South Africa	KZN Surveillance	OS	sdNVP	sdNVP	NI	NI	ViroSeq HIV-1 Genotyping system (Applied Biosystems®)	6 weeks	30	40.0
Martinson *et al.*[19]	Slides	South Africa	PMTCT program	OS	sdNVP	sdNVP	Final results	NI	Sequence analysis	6 weeks	50	48
Chaix *et al.*[7,8]	Article and unpublished technical report	Côte d'Ivoire	ANRS 1201 DITRAME Plus 1.0 and 1.1	OS	1.0: Antenatal ZDV + intrapartum ZDV + sdNVP 1.1: Antenatal ZDV + 3TC + intrapartum ZDV + 3TC + sdNVP + postpartum 3 days ZDV + 3TC	sdNVP + 1 week ZDV sdNVP + 1 week ZDV	Final results	Exhaustive sample	Sequencing reverse transcriptase gene + Sequence Navigator Software (Applied Biosystems PE®)	4 weeks	26 16	23.1 6.25
Eshleman *et al.*[14]	Article	Uganda	HIVNET 012	RCT	Intrapartum sdNVP	sdNVP	Final results	Not exhaustive sample	ViroSeq HIV-1 Genotyping system (Applied Biosystems®)	6 weeks	24	45.8
Chalermchok-charoenkit *et al.*[9]	Abstract	Thailand	Thailand CDC	OS	Antenatal ZDV + Intrapartum ZDV + sdNVP	sdNVP + 4 weeks ZDV	NI	Exhaustive sample	TruGen HIV-1 kit (Visible Genetics®)	4 weeks	10	20
Ngo-Giang-Huong *et al.*[22]	Poster	Thailand	PHPT-2/Cohort	RCT/OS	(1) Antenatal ZDV +/− sdNVP (2) Antenatal ZDV + intrapartum sdNVP	Arm 1: sdNVP + 7 days ZDV Arm 2: sdNVP + 6 weeks ZDV	NI	NI	ViroSeq HIV-1 Genotyping system (Applied Biosystems®)	6 weeks	29 21	10.3 4.5

RCT: randomised controlled trial; OS: Observational study; NI: no information; NVPR: NVP resistanc; ZDV: Zidovudine; sdNVP: single-dose Nevirapine; 3TC: Lamivudine; CBV: Combivir® (ZDV + 3TC); TOPS: Treatment Options Preservations Study; SAINT: South African Intrapartum Nevirapine Trial; NVAZ:Nevirapine-AZT (zidovudine); PMTCT: Prevention of Mother-To-Child-Transmission; KZN: KwaZulu-Natal; ANRS: Agence Nationale de Recherches sur le Sida et les hépatites virales; DITRAME: DIminution de la TRAnsmission Mere-Enfant; HIVNET: HIV Network for Prevention Trial; CDC: Center for Diseases Control; PHPT: Perinatal HIV Prevention Trial.

[a]Exhaustivity of the sample size with genotypic assessment compared with the number of participants included in the main study.

Table 5 Univariable and mutivariable meta-regression on prevalence of nevirapine (NVP) viral resistance in children at 6–8 weeks postnatally after single-dose exposure to prevent vertical transmission of HIV-1

Variables	Univariable				Multivariable					
	Random effect models				Random effect models					
	OR [CI][a]	*P*-value	OR [CI][a]	*P*-value	OR [CI][a]	*P*-value	OR [CI][a]	*P*-value	OR [CI][a]	*P*-value
Post-natal ARV intervention Yes vs No (Ref.)	0.16 [0.06–0.39]	$<10^{-3}$	0.43 [0.10–1.82]	0.219	–	–	–	–	–	–
Ante/Intra-partum ARV intervention Yes vs No (Ref.)	0.16 [0.06–0.41]	$<10^{-3}$	0.36 [0.07–1.94]	0.205	0.21 [0.05–0.94]	0.04	0.1 [0.03–0.29]	$<10^{-3}$	0.17[0.07–0.43]	0.002
Postpartum ARV intervention Yes vs No (Ref.)	0.07 [0.01–0.47]	0.010	0.15 [0.02–1.06]	0.056	0.09 [0.01–0.58]	0.016	0.05 [0.01–0.234]	0.006	0.09 [0.02–0.57]	0.015
Study location Southern Africa vs other regions (Ref.)	3.77 [1.38–10.29]	0.014	–	–	1.3 [0.35–4.84]	0.656	–	–	–	–
Exhaustivity[b] Yes vs No/No information (Ref)	0.40 [0.10–1.51]	0.158	–	–	–		2.95 [0.88–9.87]	0.074	–	–
Sample size <30 vs ⩾30 (Ref.)	0.51 [0.14–1.88]	0.282	–	–	–	–	–	–	–	–
Publication type Articles vs other types (Ref.)	1.18 [0.328–4.91]	0.810	–	–	–	–	–	–	–	–
Between-study variance	–	–	–	0.41	–	0.697	–	0.882	–	0.665

[a]Odds Ratio [Limits of 95% confidence interval]. ARV: antiretroviral.
[b]Exhaustivity of the sample size with resistance assessment compared with the number of participants included in the main study.

eligible for treatment sooner after the delivery than women with lower baseline HIV-1 RNA viral load. This was highlighted by Lockman *et al.*[25] who demonstrated that virological suppression was less frequent in women who initiated NVP-based ART <6 months from single-dose NVP exposure and among infants while this association was not found among women initiating the same kind of treatment 6 months or more from sdNVP exposure. Results from recent studies did not suggest a difference in terms of clinical or immunological response to NVP-containing ARV treatments[28,29] or efficacy of NVP-containing regimens for PMTCT in subsequent pregnancies[20,44] in women previously exposed to single-dose NVP. However, it cannot be excluded that NVP-resistant viruses following single-dose NVP exposure, even below the threshold of detection of conventional assays or archived in biological reservoirs may impair response to subsequent NNRTI-containing regimens.[24,25]

In conclusion, we confirm a substantial reduction in the prevalence of NVP resistance after single-dose NVP to prevent MTCT with the addition of a short course of dual ARV regimen (ZDV + 3TC) given to the mother post-partum. Given the high likelihood of viral resistance associated with single-dose NVP use alone, here estimated at 35.7% in mothers and 52.6% in children and the uncertain clinical implications in settings where NVP is included in first-line ARV treatment regimen, we suggest that a short-course post-partum ARV regimen should be routinely recommended.[6] This is of particular importance in settings where sub-type C is predominant. For women with high baseline HIV-1 RNA viral load and more advanced disease, fully suppressive ARV therapy, instead of single-dose NVP containing PMTCT regimens, is already recommended, partly to reduce the risk of resistance to NVP.[6] Further studies are needed to assess the association between single-dose NVP exposure, not only in the proportion of resistant mutants in plasma but also in the different compartments and subsequent response to NNRTI-based treatment in mothers and children. Furthermore, alternative regimens to prevent MTCT need urgent evaluation.

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Conflict of interest: None declared.

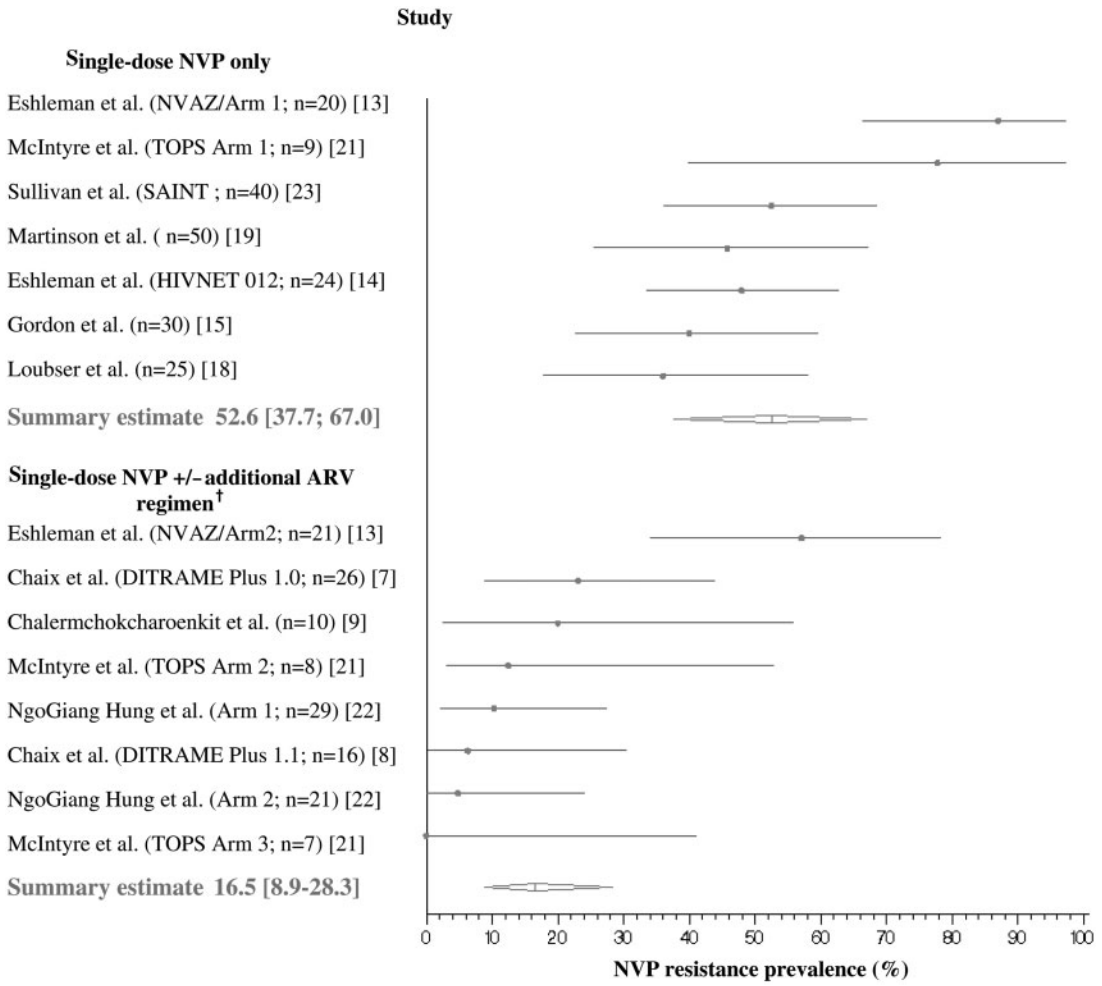

Figure 4 Forest plot of viral resistance prevalence of resistance to nevirapine (NVP) in children at 4–8 weeks postpartum after single-dose exposure, grouped according to whether mothers and children received only single-dose NVP or received additional antiretroviral therapy (univariable random effect model). †Antenatal/intrapratum zidovudine ± lamivudine and/or postpartum zidovudine and lamivudine and/or postnatal zidovudine ± lamivudine. TOPS: Treatment Options Preservations Study. SAINT: South African Intrapartum Nevirapine Trial. NVAZ:Nevirapine-AZT (zidovudine). HIVNET: HIV Network for Prevention Trial. DITRAME: DIminution de la TRAnsmission Mere-Enfant. PHPT: Perinatal HIV Prevention Trial

KEY MESSAGES

- Single-dose NVP administrated to the mother and the neonate is the most common regimen used to prevent MTCT of HIV, as it is cheap, effective and easy to use.
- However, the burden of viral resistance after single-dose NVP to prevent MTCT of HIV-1 is high in both women and children (pooled estimates of nevirapine resistance prevalence was 35.7% and 52.6%, respectively).
- This prevalence can be substantially reduced by adding short-course post-partum antiretroviral therapy to standard prophylaxis (4.5% and 16.5%).

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