

Single-dose nevirapine exposure does not affect response to antiretroviral therapy in HIV-infected African children aged below 3 years

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Objectives: To assess the impact of exposure to single-dose nevirapine (sdNVP) on virological response in young Ugandan/Zimbabwean children (<3 years) initiating antiretroviral therapy (ART), and to investigate other predictors of response.

Design: Observational analysis within the ARROW randomized trial.

Methods: sdNVP exposure was ascertained by the caregiver's self-report when the child initiated non-nucleoside reverse transcriptase inhibitor (NNRTI)-based ART. Viral load was assayed retrospectively over a median 4.1 years of follow-up. Multivariable logistic regression models were used to identify independent predictors of viral load below 80 copies/ml, 48 and 144 weeks after ART initiation (backwards elimination, exit $P=0.1$).

Results: Median (IQR) age at ART initiation was 17 (10–23) months in 78 sdNVP-exposed children vs. 21 (14–27) months in 289 non-exposed children (36 vs. 20% <12 months). At week 48, 49 of 73 (67%) sdNVP-exposed and 154 of 272 (57%) non-exposed children had viral load below 80 copies/ml [adjusted odds ratio (aOR) 2.34 (1.26–4.34), $P=0.007$]; 79 and 77% had viral load below 400 copies/ml. Suppression was significantly lower in males ($P=0.009$), those with higher pre-ART viral load ($P=0.001$), taking syrups ($P=0.05$) and with lower self-reported adherence ($P=0.04$). At week 144, 55 of 73 (75%) exposed and 188 of 272 (69%) non-exposed children had less than 80 copies/ml [aOR 1.75 (0.93–3.29), $P=0.08$]. There was no difference between children with and without previous sdNVP exposure in intermediate/high-level resistance to NRTIs ($P>0.3$) or NNRTIs ($P>0.1$) ($n=88$) at week 144.

Conclusion: Given the limited global availability of lopinavir/ritonavir, its significant formulation challenges in young children, and the significant paediatric treatment gap, tablet fixed-dose-combination NVP-based ART remains a good alternative to syrup lopinavir-based ART for children, particularly those over 1 year and even if exposed to sdNVP.

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Introduction

Despite effectively reducing mother-to-child HIV transmission, single-dose nevirapine (sdNVP) given to the mother and/or the infant at delivery has important limitations. First, the drug's long half-life, especially at birth when metabolism is limited, means sub-therapeutic levels can persist for long periods. Second, its low genetic barrier to high-level resistance caused by single point mutations favour the emergence of resistant variants in a substantial proportion of recipients; variants can also be transmitted to infants via breast milk [1].

Studies have documented poorer response to NVP-containing combination antiretroviral therapy (ART) subsequently initiated by mothers exposed to sdNVP [2,3]. The poorer virological response to NVP vs. lopinavir-containing regimens in the P1060 trials of infants exposed [4], and non-exposed [5], to sdNVP led WHO to recommend universal ART initiation with lopinavir/ritonavir-containing regimens in children aged below 3 years [6]. However, further analysis of pooled P1060 data [7] found no impact of sdNVP on a composite endpoint of viral load failure (>400 copies/ml at week 24 or >4000 copies/ml subsequently) or death, which occurred in 13 of 84 (19%) sdNVP-exposed (median age 8 months; $CD4^+%$ 19%) vs. 30 of 145 (21%) non-exposed (20 months; 15%) children initiating NVP-based ART. Other evidence supporting poorer response to NVP-containing regimens in sdNVP-exposed infants is limited. One small study found virological failure by 6 months in 10 of 15 sdNVP-exposed infants vs. one of 15 non-exposed infants (median age 1 month at initiation of NVP-based ART) [8]. Another found only 38% of 35 sdNVP-exposed Ugandan children (median age 6 months; $CD4^+%$ 16%) vs. 68% of 69 non-exposed children (22 months; 12%) had viral load below 400 copies/ml, 48 weeks after initiating non-nucleoside reverse transcriptase inhibitor (NNRTI)-based ART [9], but did not estimate associations adjusted for receipt of NVP vs. efavirenz (respectively, 97 vs. 3% sdNVP-exposed, 71 vs. 29% non-exposed). In contrast, another Ugandan study found 76% of 44 sdNVP-exposed children (median age 20 months; $CD4^+%$ 14%) vs. 80% of 48 non-exposed children (median 7.8 years; 8%) had viral load below 400 copies/ml, 48 weeks after ART initiation with NVP-based regimens [10].

The WHO guidelines now recommend all children aged below 5 years initiate ART regardless of immune or clinical status, and that those aged below 3 years initiate protease inhibitor-based regimens. However, lopinavir/ritonavir availability is limited, and for young children, the only current formulation is an unpalatable liquid with cold-chain requirements, providing management challenges at lower-level health facilities. When first-line lopinavir-containing ART is not feasible, WHO 2013 guidelines suggest NNRTI-based regimens should be

initiated as an alternative, because mortality in untreated young children is very high; the NNRTI of choice is NVP, because dosing of efavirenz is challenging in young children [11]. Understanding whether sdNVP is associated with substantially greater risks of virological failure in children initiating NVP-based ART aged above 1 month of age therefore continues to have programmatic relevance, particularly in sub-Saharan Africa, where most HIV-infected children live and where rollout of universal combination ART for pregnant women (option B+) is gathering pace. Furthermore, a substantial proportion of African women still have no or incomplete antenatal care and deliver their babies at home, when the risk of receiving no interventions at all for prevention of mother-to-child transmission (PMTCT) remains high. We therefore compared viral load response between children initiating NVP-based ART aged below 3 years with and without previous sdNVP exposure in the AntiRetroviral Research fOR Watoto (ARROW) trial.

Methods

Analyses included 367 previously untreated (except for PMTCT) Ugandan/Zimbabwean children initiating NVP-based ART aged 3 months to less than 36 months in the ARROW trial (ISCRTN24791884). Three children aged below 36 months (32, 35 and 35 months) initiated efavirenz-based ART and were excluded. The trial recruited from March 2007 to November 2008: before this, and during recruitment, sdNVP to the mother and child was the national PMTCT strategy. ART taken by the mother during pregnancy, delivery or breastfeeding, and (separately) ART taken by the child were determined by self-report at enrolment.

The children were randomized 1:1 to clinically-driven monitoring vs. laboratory + clinical monitoring for toxicity (haematology/biochemistry) and efficacy ($CD4^+$). Children were also randomized 1:1:1 in a factorial design to open-label lamivudine + abacavir + NNRTI continuously (arm A, no zidovudine) vs. induction-maintenance with four-drug lamivudine + abacavir + NNRTI + zidovudine for 36 weeks, then either lamivudine + abacavir + NNRTI (arm B; short-term zidovudine) or lamivudine + abacavir + zidovudine (arm C; long-term zidovudine). Children were recruited from three centres in Uganda and one in Zimbabwe. All children were examined by a doctor at screening, randomization, weeks 4, 8, and 12, and then every 12 weeks. Every 4–6 weeks, the children were reviewed by a nurse, and adherence was assessed using a questionnaire completed by the carer. The trial was approved by Research Ethics Committees in Uganda, Zimbabwe and the United Kingdom. The caregivers gave written consent.

The viral load was assayed retrospectively on stored plasma samples at 0, 4, 24, 36, 48 and 144 weeks post-

ART initiation, and the last study visit before trial closure on 16 March 2012 in all children. The viral load was additionally assayed 24-weekly after week 48 in children enrolled after June 2008 (immunology/virology sub-study); and in an overlapping subset at, and 48 and 96 weeks after, a subsequent randomization to once vs. twice-daily lamivudine + abacavir (which were virologically equivalent [12]). Assays were run using Abbott m2000rt (in Uganda; Abbott, Maidenhead, UK) and Roche Amplicor 1.5 (in Zimbabwe; Roche, Basel, Switzerland). The closest measurement to 4, 24, 36 and 48 weeks on ART, and then 24-weekly (in equally spaced windows), was used in analyses, which used a lower detection limit of 80 copies/ml because many low-volume samples had to be diluted 1:2. Samples with more than 1000 copies/ml at week 48 or 144, or any time point in the once/twice-daily study, were genotyped (reverse transcriptase only). The closest genotype to week 144 from week 48 through to trial end was used for analysis. Major nucleoside reverse transcriptase inhibitor (NRTI) mutations were defined according to International AIDS Society (IAS) 2013 [13], and drug susceptibility was predicted using the Stanford algorithm version 7 [10].

Pre-ART characteristics of sdNVP-exposed and non-exposed children were compared using chi-square tests for categorical factors and Wilcoxon rank-sum tests for continuous values. Predictors of suppression below 80 copies/ml, 48 and 144 weeks after ART initiation, were identified using logistic regression (backwards elimination; exit $P=0.1$ to develop an explanatory model), forcing into the models sdNVP (the primary exposure), age at ART initiation (a major known confounder) and ART-strategy randomization [because, at week 144, triple NRTI maintenance (arm C) was virologically inferior to 2NRTI + NNRTI (arms A and B) in the trial as a whole] [14]. The 80 copies/ml threshold was chosen to provide the most sensitive investigation of the possible impact of low-level resistant variants following sdNVP exposure. Other factors considered were pre-ART WHO stage, CD4⁺%, weight/height-for-age Z-scores (WHO reference [15]) and viral load; sex, trial centre, CD4⁺ monitoring randomization; current or initial ART taken as all syrups; and whether the caregiver reported missed ART doses (in the last 4 weeks; percentage of scheduled visits in the last 48 weeks). Missing data were very few, so models included complete cases only. Non-linearity in the effects of continuous predictors was explored using natural cubic splines, with three knots at the 10th, 50th and 90th centiles [16]. Interactions between variables included in final models were investigated when heterogeneity P was less than 0.05. In additional main-effect models, the primary caregiver (mother/other) and socioeconomic variables at ART initiation (physical house structure, electricity, household assets) were also included. As children in arm C stopped NNRTI at week 36, secondary analyses considered only arms A and B receiving long-

term NNRTI. All analyses were performed using Stata 13.1 (StataCorp, College Station, Texas, USA). All P values were two-sided.

Results

Among the 367 children aged 3 to below 36 months initiating NVP-based ART, 78 (21%) had received sdNVP (Supplementary Fig. 1, <http://links.lww.com/QAD/A715>): 51 to both the mother and child, 4 to the child alone, 20 to the mother alone (administration to child may not have been recorded) and 3 when the specific regimen was unknown (assumed to be sdNVP). Additional zidovudine was not recorded as received in any of these children, likely reflecting their age at enrolment, given that WHO 2006 PMTCT guidelines (including 1 week zidovudine [17]) were adopted during 2008. The mother was more likely to be the primary caregiver of children who had received sdNVP (99 vs. 78% non-exposed; $P<0.001$). Children receiving sdNVP were younger at ART initiation (median 17 vs. 21 months, $P=0.0008$; 36 vs. 20% <12 months) and therefore had slightly higher CD4⁺ cell counts (914 vs. 704 cells/ μ l; $P=0.003$), but did not differ significantly in pre-ART CD4⁺% (median 14%), weight-for-age Z-score (-2.2) and other pre-ART characteristics (Table 1).

Among the 367 children, 350 (95%) were alive and in follow-up 48 weeks after ART initiation, with viral load measurements available in 345 of 350 (99%) (Supplementary Fig. 1, <http://links.lww.com/QAD/A715>). Fourteen children had died and three had been lost. At 48 weeks, sdNVP-exposed children were more likely to receive ART as all syrups vs. any tablets (73 vs. 57% in non-exposed; $P=0.02$) and less likely to have missed doses in the past 4 weeks (1 vs. 9%; $P=0.04$) (Table 1). One hundred and forty-four weeks after ART initiation, 346 children (94%) were alive and in follow-up, 345 with viral load available (four lost to follow-up since 48 weeks). Only 10 of 367 (3%) children switched to protease inhibitor-containing regimens during follow-up – 1 for toxicity (week 14; hepatitis on NVP) and 9 for first-line clinical/immunologic failure [median 153 weeks, range 88–253; 2 (3%) sdNVP-exposed, 7 (2%) non-exposed]. Overall 95.5 and 94.5% of child-time through 48 weeks was spent on NVP-containing ART in sdNVP-exposed and non-exposed children, and 91.8 and 92.6% through 144 weeks, respectively [only including children randomized to long-term NVP-containing regimens (arms A and B) from week 36 onwards]. Most first-line NVP substitutions were to efavirenz for tuberculosis co-treatment or rash. In sdNVP-exposed and non-exposed children, 84.3 and 79.9% of child-time through 48 weeks was spent receiving ART as all syrups vs. any tablets, and 37.9 and 21.1% from 48 to 144 weeks.

Overall, there was no evidence that suppression below 80 copies/ml was any poorer in sdNVP-exposed vs.

Table 1. Characteristics of single-dose nevirapine-exposed and non-exposed children at antiretroviral therapy initiation and 48 and 144 weeks later.

	sdNVP (<i>n</i> = 78)	No sdNVP (<i>n</i> = 289)	<i>P</i> ^a
Male	43 (55%)	134 (46%)	0.17
At ART initiation			
Age (months)			
Median (IQR)	17 (10–23)	21 (14–27)	0.0008
3 to <6 months	1 (1%)	3 (1%)	
6 to <12 months	27 (35%)	54 (19%)	
12 to <24 months	34 (44%)	120 (42%)	
24 to <36 months	16 (21%)	112 (39%)	
CD4 ⁺ (cells/ μ l): median (IQR)	914 (658–1337)	704 (475–1101)	0.003
CD4 ⁺ %	15 (11–20)	14 (10–19)	0.31
Weight-for-age Z-score: median (IQR)	–2.1 (–3.4 to –1.1)	–2.3 (–3.5 to –1.4)	0.53
Weight (kg): median (IQR)	7.8 (6.4–10.0)	8.5 (7.0–10.0)	0.08
Height-for-age Z-score: median (IQR)	–2.9 (–4.0 to –2.1)	–2.9 (–3.8 to –2.0)	0.77
VL (copies/ml): median (IQR)	757100 (192100–2076700) ^b	476400 (184500–1253100) ^c	0.18
WHO stage 3/4	57 (73%)	204 (71%)	0.84
Randomized treatment strategy			0.20
Arm A (3TC/ABC/NNRTI throughout)	21 (27%)	98 (34%)	
Arm B (3TC/ABC/NNRTI throughout, ZDV until week 36)	31 (40%)	85 (29%)	
Arm C (3TC/ABC/ZDV throughout, NNRTI until week 36)	26 (33%)	106 (37%)	
Initial ART as all syrups	74 (95%)	272 (94%)	0.80
Allocated monitoring strategy			0.27
Routine CD4 ⁺ monitoring	32 (41%)	139 (48%)	
No CD4 ⁺ monitoring	46 (59%)	150 (52%)	
Country/centre			0.39
Uganda/Entebbe	5 (7%)	37 (13%)	
Uganda/JCRC	19 (24%)	67 (23%)	
Uganda/PIDC	33 (42%)	123 (43%)	
Zimbabwe/Harare	21 (27%)	62 (21%)	
Primary carer			<0.001
Mother	77 (99%)	224 (78%)	
Other	1 (1%)	64 (22%)	
Missing ^d	0	1	
House structure			0.57
Poor	15 (19%)	43 (15%)	
Adequate	17 (22%)	58 (20%)	
Good	45 (58%)	183 (64%)	
Missing ^d	1	5	
Electricity			0.07
No	19 (25%)	103 (36%)	
Yes	58 (75%)	185 (64%)	
Missing ^d	1	1	
Affluence score: mean ^e	2.6	2.5	0.43
Week 48: <i>N</i> alive and in follow-up	74	276	
Current ART as all syrups	54 (73%)	158 (57%)	0.01
Missed doses in last 4 weeks	1 (1%)	25 (9%)	0.04
% visits to date with missed doses in last 4 weeks: mean	7.9	9.9	0.15
Week 144: <i>N</i> alive and in follow-up	73	273	
Current ART as all syrups	5 (7%)	10 (4%)	0.24
Missed doses in last 4 weeks	2 (3%)	19 (7%)	0.18
% visits in last 48 weeks with missed doses in last 4 weeks: mean	6.6	8.3	0.46

3TC, lamivudine; ABC, abacavir; ART, antiretroviral therapy; IQR, inter-quartile range; JCRC, Joint Clinic Research Centre, NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; NVP, nevirapine; PIDC, Paediatric Infectious Diseases Clinic; sdNVP, single-dose nevirapine; ZDV, zidovudine

^aChi-square tests for categorical factors and Wilcoxon rank-sum tests for continuous values unless otherwise indicated.

^b*n* = 76 (2 missing baseline VLs).

^c*n* = 288 (1 missing baseline VL).

^dMode assumed in multivariate analyses.

^eNumber of the following items in the house: fridge, radio, television, landline, mobile, motorbike, bicycle, car. Missing for 1 child in the sdNVP-non-exposed group, mode assumed in multivariate analyses.

non-exposed, with similar results for less than 400 and less than 1000 copies/ml ($P > 0.1$; Fig. 1). Mean viral load reduction from baseline to week 4 was 2.5 and 2.4 log₁₀ in sdNVP-exposed and non-exposed, respectively {unadjusted difference +0.1 [95% confidence interval (CI) –0.1, +0.3], $P = 0.41$, $n = 339$ }.

At week 48, 49 of 73 (67%) sdNVP-exposed and 154 of 272 (57%) non-exposed children were below 80 copies/ml [adjusted odds ratio (aOR) 2.34 (1.26–4.34), $P = 0.007$, $n = 342$ complete cases], indicating, if anything, better suppression with sdNVP exposure. At week 144, 55 of 73 (75%) exposed and 188 of 272 (69%)

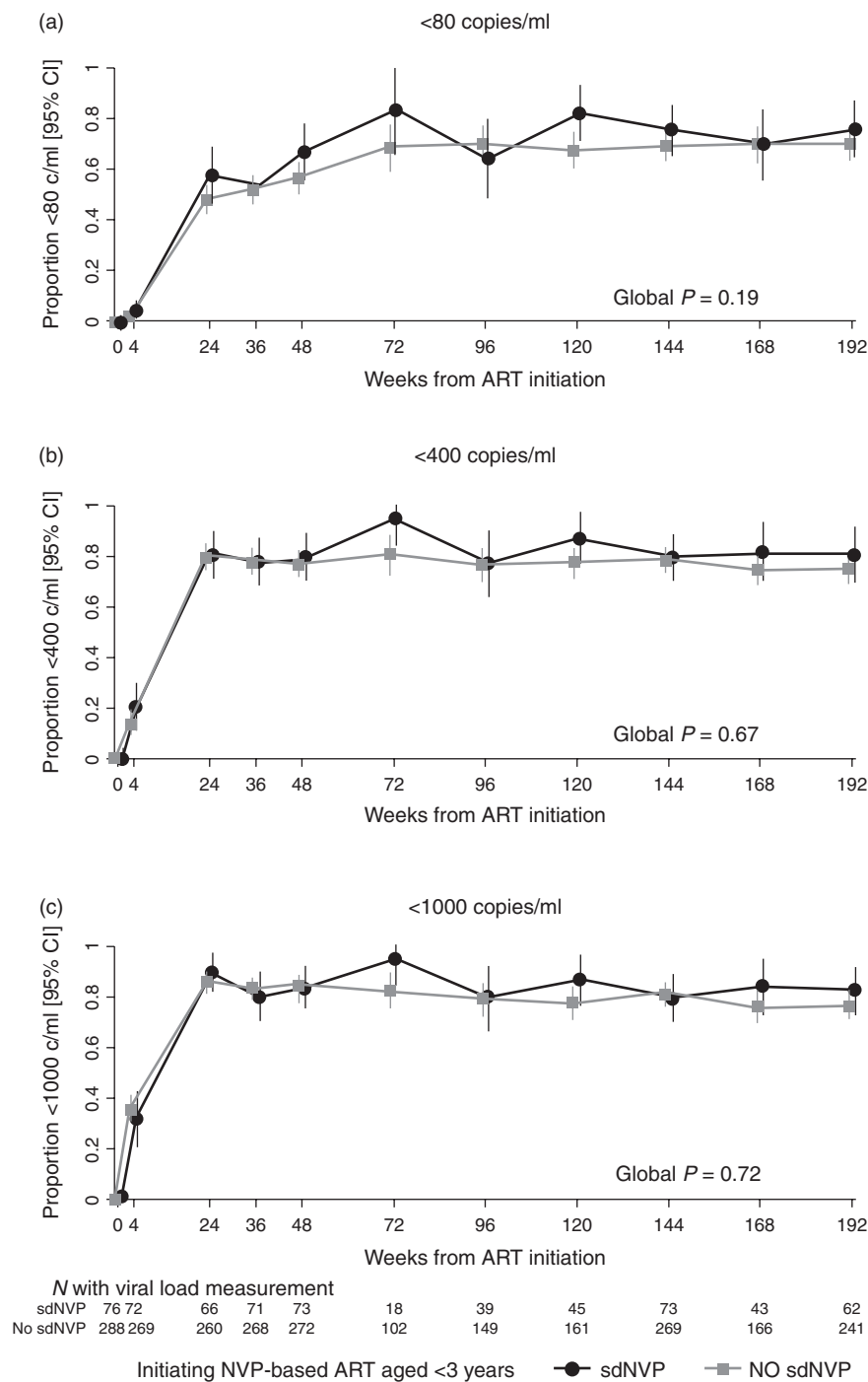


Fig. 1. Suppression over time. (a) Below 80, (b) below 400 and (c) below 1000 copies/ml.

non-exposed were below 80 copies/ml [aOR 1.75 (0.93–3.29), $P = 0.08$, $n = 343$ complete cases].

At week 48, suppression below 80 copies/ml was lower in males ($P = 0.009$), those with higher pre-ART viral load ($P = 0.001$), currently taking all ART as syrups ($P = 0.05$) and whose caregivers reported lower adherence ($P = 0.04$) (Table 2). Suppression was non-significantly poorer in children who were younger at ART initiation

($P = 0.11$). Initiating ART with all syrups vs. any tablets (rather than week 48 formulation) was not associated with suppression at week 48 ($P = 0.80$). There was no evidence of interaction between sdNVP and age ($P = 0.70$) or any other factors in the final model ($P > 0.2$), or between these factors and ART-strategy randomization ($P > 0.3$).

At week 144, suppression remained lower in children who were younger at ART initiation ($P = 0.09$) and those with

Table 2. Independent predictors of viral load below 80 copies/ml 48 and 144 weeks after antiretroviral therapy initiation.

	Week 48 (N = 342) OR (95% CI)	P	Week 144 (N = 343) OR (95% CI)	P
Main models				
sdNVP exposure (yes vs. no)	2.34 [1.26–4.34]	0.007	1.75 [0.93–3.29]	0.08
Age at ART initiation (per year younger)	0.70 [0.46–1.08]	0.11	0.72 [0.50–1.05]	0.09
Allocated treatment strategy, vs. arm A (3TC/ABC/NNRTI throughout)		0.20		0.22 ^a
Arm B (3TC/ABC/NNRTI throughout, ZDV until week 36)	0.77 [0.43–1.38]	0.38	0.78 [0.42–1.44]	0.43
Arm C (3TC/ABC/ZDV throughout, NNRTI until week 36)	1.30 [0.74–2.31]	0.36	0.60 [0.33–1.07]	0.08
Pre-ART VL (per log ₁₀ higher)	0.55 [0.38–0.79]	0.001	0.57 [0.39–0.83]	0.003
Male (vs. female)	0.53 [0.33–0.85]	0.009		0.10 ^b
Current ART as all syrups (vs. tablets)	0.56 [0.31–1.01]	0.05		0.13 ^c
Missed doses in last 4 weeks (yes vs. no)	0.35 [0.13–0.94]	0.04		0.92

Note: multivariable models based on backwards elimination (exit $P = 0.1$) on complete cases from all factors in Table 1, forcing sdNVP, age at ART initiation and ART-strategy randomization into the model. *Italic data show effect from adding variables into the final model.* No evidence of nonlinearity in age at week 48 ($P = 0.9$) or 144 ($P = 0.6$). 3TC, lamivudine; ABC, abacavir; ART, antiretroviral therapy; CI, confidence interval; IQR, inter-quartile range; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; NVP, nevirapine; OR, odds ratio; sdNVP, single-dose nevirapine; VL, viral load; ZDV, zidovudine.

^aArm C vs. A and B combined [OR 0.67 (0.41–1.10), $P = 0.12$].

^bAdjusted (for factors above) [OR 0.67 (0.41–1.08), $P = 0.10$].

^cAdjusted (for factors above) [OR 0.40 (0.12–1.33), $P = 0.13$]; only 4% children not taking at least one drug as tablet formulation at week 144.

higher pre-ART viral load ($P = 0.003$). The effect of sex was in the same direction as at week 48, but non-significant ($P = 0.10$). Suppression was also non-significantly lower in children who were on maintenance with triple NRTI (arm C) vs. 2NRTI + NNRTI (arms A and B) ($P = 0.12$). Almost all children (96%) were receiving ART as tablets by week 144, reducing power to detect effects of syrups which were in the same direction as week 48 ($P = 0.13$). There was no evidence that missing ART doses in the last 4 weeks ($P = 0.92$) or the proportion of follow-up visits in the last 48 weeks reporting missed doses in the last 4 weeks ($P = 0.71$) affected suppression. Considering interactions, there was some evidence that the (if anything) slightly better suppression in sdNVP-exposed children was greater at lower pre-ART viral loads, with little difference in children with pre-ART viral load above 1000 000 copies/ml (interaction $P = 0.04$) (Supplementary Table 1, <http://links.lww.com/QAD/A715>). Although this interaction was not statistically significant at 48 weeks ($P = 0.26$), results were qualitatively similar. There was also some evidence that the lower suppression in those who were younger at ART initiation was restricted to those on maintenance with 2NRTI + NNRTI (arms A and B) vs. triple NRTI (arm C) (interaction $P = 0.01$; Supplementary Table 1). This interaction was not apparent at 48 weeks ($P = 0.88$). There was no evidence of interaction between sdNVP and age ($P = 0.63$) or any other factors retained in the final model ($P > 0.6$), and there were no other statistically significant interactions between ART-strategy randomization and any factors in the final model ($P > 0.05$).

In subsequent models, the primary caregiver (mother/other) and socioeconomic variables were also included as potential confounders between sdNVP and viral load suppression. Suppression below 80 copies/ml was greater in children in households that were more affluent at ART initiation [week 48: aOR 1.14 per affluence point

(defined in Table 1) (0.98–1.32), $P = 0.10$; week 144: 1.19 (1.01–1.39), $P = 0.04$]. Suppression at week 144 was also independently greater in households with electricity [aOR 1.65 (0.99–2.74), $P = 0.05$]. There was no evidence of any independent effects of caregiver or other socioeconomic factors at either time point ($P > 0.2$), and no evidence that the slightly better suppression with sdNVP was mediated by any of these factors (estimated aOR for sdNVP-exposed vs. non-exposed > 1.6 across all models at weeks 48 and 144).

Results were broadly similar categorizing sdNVP as received by both mother and child, child alone or mother alone (when administration to child may not have been recorded) [week 48 aOR vs. no sdNVP: 2.27 both, 1.47 child alone ($n = 4$), 2.83 mother alone (heterogeneity $P = 0.86$); week 144: 1.85, 0.67, 1.97, respectively (heterogeneity $P = 0.63$)]. Results were also similar restricting to children on long-term NNRTI (arms A and B).

Eighteen (23%) sdNVP-exposed vs. 70 (24%) non-exposed children had an available genotype, a median [inter-quartile range (IQR) (range)] 144 [133–147 (48–228)] weeks from ART initiation, respectively (rank-sum $P = 0.55$). Fourteen (78%) vs. 48 (69%) children, respectively, had one or more IAS major NNRTI mutations (median 1 vs. 1, respectively, per child; $P = 0.85$) and 17 (94%) vs. 63 (90%), respectively, had one or more IAS NRTI mutations (median 3 vs. 3, respectively, per child; rank-sum $P = 0.74$; Fig. 2). Median viral load at the genotype was 4800 vs. 16700 copies/ml, respectively ($P = 0.17$). There was no evidence of difference between children with and without previous sdNVP exposure in the percentage with intermediate/high-level resistance to any NRTIs ($P > 0.3$) or NNRTIs ($P > 0.1$) (Fig. 3). Of the nine children who switched for first-line failure, 5/5 on maintenance with 2NRTI + NNRTI (arms A and B) vs.

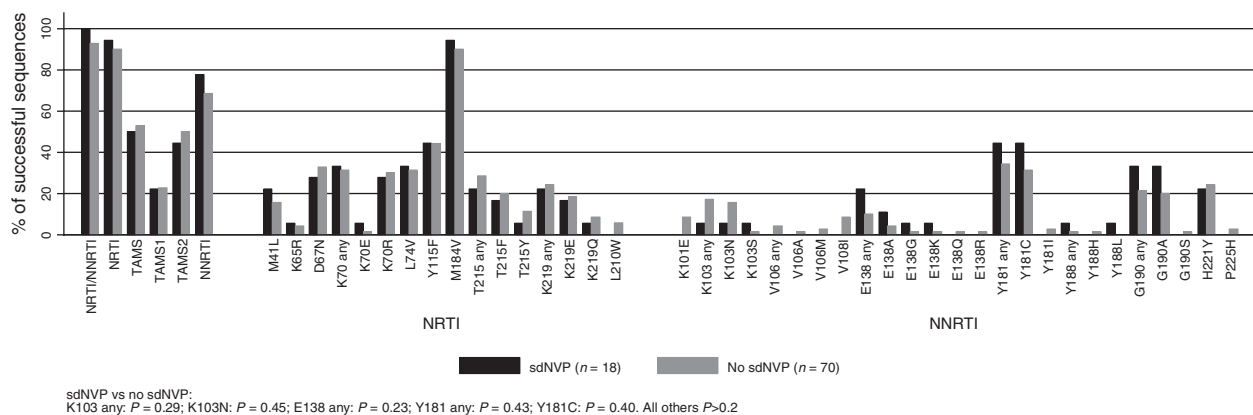


Fig. 2. Prevalence of major IAS drug-resistance mutations by single-dose nevirapine exposure. sdNVP vs. no sdNVP: K103 any: $P = 0.29$; K103N: $P = 0.45$; E138 any: $P = 0.23$; Y181 any: $P = 0.43$; Y181C: $P = 0.40$. All others $P > 0.2$. sdNVP, single-dose nevirapine.

2/4 triple NRTI (arm C) had one or more IAS major NNRTI mutations at switch (median 2 vs. 0.5, respectively, per child; $P = 0.01$).

Discussion

Although WHO guidelines recommend all HIV-infected infants and young children aged below 3 years initiate ART with lopinavir-containing regimens [6], the only licensed lopinavir formulation in this age group is an oral solution, which is expensive, requires cold chain, contains a high percentage of ethanol and is contraindicated in premature/very young infants. A sprinkle 'pellet' formulation is not yet licensed or commercially available, and caregivers still had major problems with its taste in children aged 1–4 years [18]. Practically, therefore, particularly outside large urban centres, the decision facing many healthcare workers is whether to initiate ART with a non-lopinavir-containing regimen or not treat the infant/child at all. The latter leads to very high risks of early mortality and morbidity [11]. The former almost invariably means a NVP-based regimen, given the challenges of efavirenz dosing in young children. Here, we have shown in a relatively young cohort without severe immunodeficiency (median age 18 months, almost all ≥ 6 months; median CD4⁺% 14%) that prior self-reported sdNVP receipt is not associated with poorer virological response to NVP-containing ART. This was similar for younger and older children in the cohort. Our findings are consistent with one of the two previous Ugandan studies, where the non-sdNVP-exposed cohort was considerably older and more immunosuppressed [10], and the P1060 cohort [7]. Furthermore, we found that sdNVP exposure was not associated with increased NRTI or NNRTI resistance accompanying viral load above 1000 copies/ml on ART. In the other studies to observe differences in viral load response, NVP-based treatment was initiated closer to birth (median 1 and 6 months of age) [8,9]. The WHO 2013 PMTCT guidelines now recommend

universal triple ART to all pregnant and breastfeeding women, and a 6-week course of daily NVP to the infant [6]. This might put those infected despite PMTCT at greater risk of developing resistance than previously.

We adjusted for potential confounders including age at ART initiation, ART-strategy randomization and also socioeconomic variables at ART initiation. It is therefore unclear why suppression remained slightly better with sdNVP exposure, possibly due to chance. As expected, high pre-ART viral load strongly predicted poorer virological suppression at both 48 and 144 weeks. Interestingly and importantly, however, the impact of receiving ART with all syrups vs. any tablets was equivalent to initiating ART with a 1 log₁₀ higher viral load. This impact of receiving ART with all syrups vs. any tablets was also of similar magnitude to the difference in viral load response between lopinavir-containing vs. NVP-containing regimens in P1060, where all children received syrups/solutions [7]. As triple-drug NVP-based fixed-dose combination (FDC) tablets are available for children weighing from 3 kg [19], this suggests that a tablet NVP-based regimen might have similar virological responses to a syrup lopinavir-based regimen in young infants/children. This may be particularly the case if NVP dose escalation is not used in these young children who have considerably faster NVP clearance than older children, and where initiating NVP at full dose led to similar plasma levels 2 weeks after ART initiation as older children initiating with half-dose [20]. A strategy of initiating ART with full-dose NVP has been shown to be well tolerated and effective in children, with 78% below 250 copies/ml 96 weeks after ART initiation and no NVP reactions among children aged below 2 years [21]. A cross-over pharmacokinetic sub-study demonstrated significantly lower lamivudine plasma levels with syrup vs. tablet administration [22] in young children; whether this could also contribute to poorer viral load response with syrups is unclear. Caregivers, and children able to express a preference, strongly prefer tablet formulations

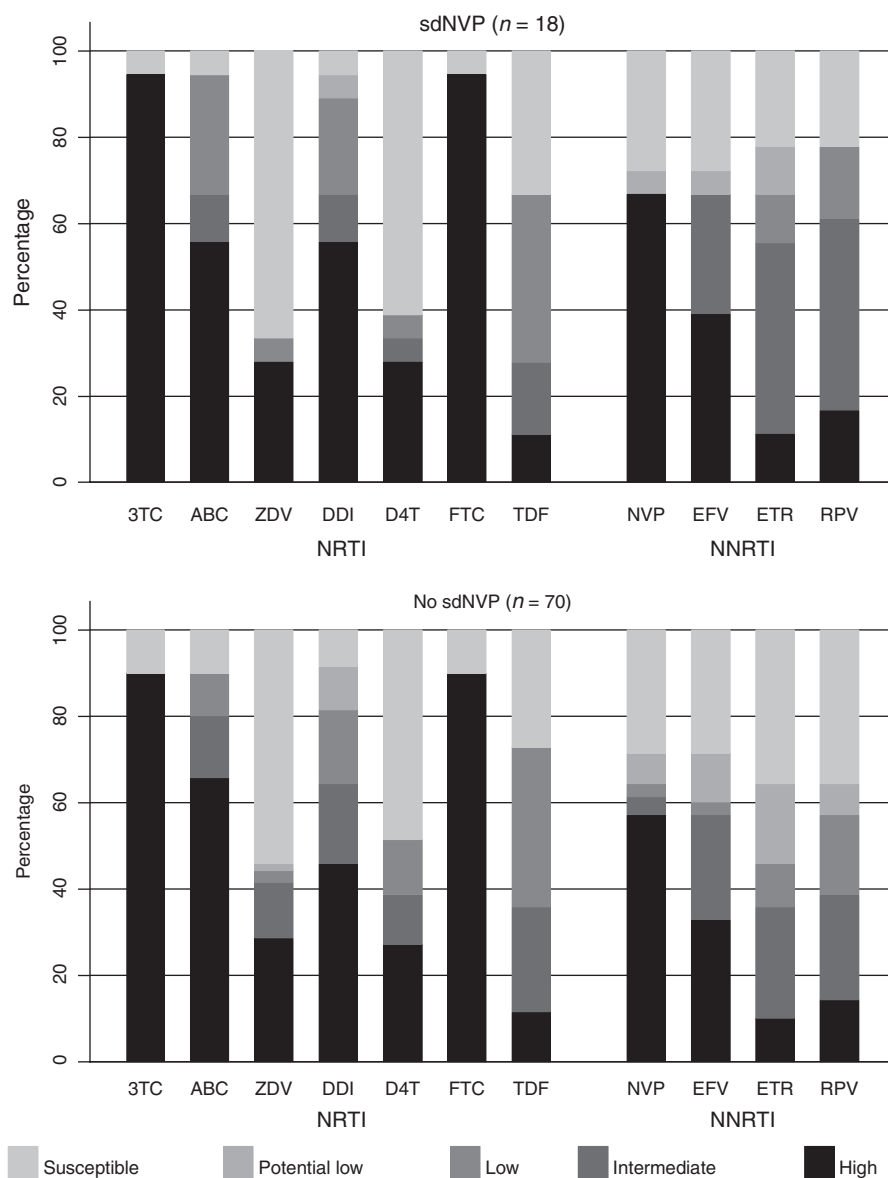


Fig. 3. Overall resistance to nucleoside reverse transcriptase inhibitor and non-nucleoside reverse transcriptase inhibitor drugs in children with and without previous single-dose nevirapine exposure. 3TC, lamivudine; ABC, abacavir; D4T, stavudine; DDI, didanosine; EFV, efavirenz; ETR, etravirine; FTC, emtricitabine; NVP, nevirapine; RPV, rilpivirine; TDF, tenofovir; ZDV, zidovudine.

for multiple reasons, including the number, weight, transportation and conspicuousness of the syrup [23].

Caregivers administered all drugs, so it is unclear why males had poorer viral load suppression; studies have sometimes [24], but not always [25], found this in older children, but it has typically been ascribed to better adherence and health behaviour in girls which is not relevant to this young cohort. We also found some suggestion that younger age (<3 years) was associated with poorer short-term virological suppression independently of pre-ART viral load, formulation, adherence and sex; in the longer term, this was restricted to those on maintenance with 2NRTI + NNRTI vs. triple NRTI. In the ARROW trial as a whole, we previously

demonstrated viral load responses were as good in children aged below 3 years as in those aged above 3 years [14]. This illustrates the substantial variation with age that categorization can mask, given the specific and numerous challenges in medication administration as infants become toddlers, and then small children.

Although approximately a third of the children were randomized to 3NRTI + NNRTI for 36 weeks then 3NRTI (arm C), any inferior viral load response during these first 36 weeks would likely be reflected in the longer term, and so the primary analyses included all children. However, the results were similar restricting to those on NNRTI-containing regimens in the long term. Another study limitation is that sdNVP exposure was based on

self-report, in contrast to previous trials in which medical records/health cards were reviewed [4,5]. Baseline genotypes based on either bulk or minority sequence are not available, so we were unable to investigate this further. However, given the young age of the cohort at recruitment in 2007–2008, it is plausible that self-report was reasonably accurate, as it would not have required substantial recall, although whether sdNVP was administered to the mother, child or both may be less accurate. In children receiving sdNVP, the primary caregiver was more likely to be the mother, unlikely to be explained by the slightly younger age of the sdNVP group, suggesting other caregivers might not have been aware of sdNVP use. However, self-report is undoubtedly what would be used in programmes. Although we focused on suppression below 80 copies/ml, arguing that this would provide the most sensitive test of the impact of minority NNRTI-resistant variants, results were similar using higher viral load thresholds of 400 and 1000 copies/ml (data not shown). The fact that it took approximately 72 weeks on ART for these young children to fully suppress to less than 80 copies/ml, despite most being less than 400 copies/ml by 24 weeks, with very few treatment changes, highlights the importance of evaluating virological suppression over the longer term in children.

Given our findings, and no detrimental effect of sdNVP exposure on subsequent response to NVP-based ART in most other paediatric studies, tablet-FDC NVP-based ART continues to be a good alternative to syrup lopinavir-based ART for children of all ages, particularly when protease inhibitor regimens are not feasible and in those over 1 year, and even if exposed to sdNVP. Concerns about sdNVP exposure may reduce over the coming years now immediate ART initiation is recommended once HIV infection is identified in infants [6]. However, the significant treatment gap, with only 34% of children in need receiving ART [26], suggests treating young children will likely remain a significant challenge. The wide availability of triple-drug NVP-based FDCs is an additional advantage, given the limited global availability of lopinavir/ritonavir and its significant formulation challenges in young children. This message is particularly important for ART roll-out to primary health facilities which is a priority for all African countries and requires that healthcare workers test and treat children alongside adults.

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Conflicts of interest

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