HIV Resistance and Prevention of Mother-to-Child Transmission Regimen in HIV-Infected Infants in Northern Tanzania

Dorothy E. Dow,¹⁻³ Werner Schimana,⁴ Balthazar M. Nyombi,^{2,5} Blandina T. Mmbaga,² Aisa M. Shayo,² John A. Bartlett,^{2,3,6} Charles G. Massambu,⁷ Emmanuel G. Kifaró,^{2,5} Elizabeth L. Turner,^{3,8} Todd DeMarco,⁹ Fangping Cai,⁹ Coleen K. Cunningham,^{1,3} and Ann M. Buchanan¹⁰

Abstract

Prevention of mother-to-child transmission (PMTCT) guidelines recommend that all HIV-infected pregnant women receive antiretroviral therapy (Option B) and HIV-infected infants should initiate therapy with a protease inhibitor-based regimen; however, implementation of these guidelines has lagged in many resource-limited settings. Tanzania only recently implemented these guidelines with little country-specific data to inform whether HIV non-nucleoside reverse transcriptase inhibitor (NNRTI) resistance was present among infected infants under the Option A guidelines. This study aimed to identify primary resistance mutations in HIV-infected infants and to identify risk of nevirapine (NVP) resistance based on maternal and infant NVP exposure. Infant dried blood spots (DBSs) were sent to the zonal reference laboratory at Kilimanjaro Christian Medical Centre Clinical Laboratory and underwent polymerase chain reaction testing for HIV as standard of care. Using the clinical laboratory registry, HIV-positive DBS cards, stored at ambient temperature, were identified and sent for further viral load testing, nucleotide sequencing, and analysis. Clinical information was obtained from the PMTCT clinical sites and the National PMTCT registry for information regarding maternal and infant demographics and PMTCT treatment regimen. Results demonstrated that infants exposed to NVP were more likely to have high level resistance mutations (HLRMs) to NVP than those infants not exposed to NVP ($p = .002$). The most common HLRMs to NVP were K103N, Y181C, and Y188L. HIV subtype A was most common, followed by subtype C. Approximately one-third of HIV-infected infants had documented referral to HIV care. This study demonstrated the ongoing need to scale up and strengthen points along the PMTCT continuum and supported the recommendation for all HIV-infected infants to initiate a lopinavir/ritonavir-based antiretroviral therapy regimen.

Keywords: HIV, nevirapine resistance, PMTCT, option B+, early infant diagnosis, dried blood spot

Introduction

DESPITE THE SUCCESSES of prevention of mother-to-child transmission (PMTCT) of HIV, gaps persist in the PMTCT cascade. Globally, there were an estimated 150,000 children diagnosed with HIV in 2015, the majority of whom

live in sub-Saharan Africa.¹ The World Health Organization now recommends that all HIV-infected pregnant women receive antiretroviral therapy (ART), regardless of CD4 count or clinical stage (Option B); or ART for life (Option B+)²; however, implementation of this recommendation has been delayed in many resource-poor settings.

¹Division of Infectious Diseases, Department of Pediatrics, Duke University Medical Center, Durham, North Carolina.

²Kilimanjaro Christian Medical Centre, Moshi, Tanzania.

³Duke Global Health Institute, Duke University, Durham, North Carolina.

⁴Department of Health Promotion for Small Children, Municipality of Munich, Munich, Germany.

⁵Kilimanjaro Christian Medical Center Clinical Laboratory, Duke University Medical Center, Durham, North Carolina.

⁶Division of Infectious Diseases and International Health, Department of Medicine, Duke University Medical Center, Durham, North Carolina.

⁷Ministry of Health and Social Welfare, Dar es Salaam, Tanzania.

⁸Department of Biostatistics and Bioinformatics, Duke University, Durham, North Carolina.

⁹Duke Human Vaccine Institute, Duke Medical Center, Durham, North Carolina.

¹⁰ViiV Healthcare, Research Triangle Park, North Carolina.

At present, pregnant women in Kilimanjaro and Arusha Regions of Tanzania receive Option B+ (tenofovir, lamivudine, efavirenz once daily fixed-dose combination pill); however, until February 2014, Option A was standard of care. Option A consisted of antepartum daily zidovudine (AZT) as early as 14 weeks of gestation, followed by single-dose nevirapine (sdNVP) at onset of labor, and twice daily AZT plus lamivudine (3TC) for the mother for 7 days postpartum. Breastfeeding prophylaxis for infants consisted of sdNVP at birth with daily NVP until 1 week after breast milk cessation. For non-breastfeeding children, the recommendation was NVP daily until 6 weeks of age.

Because sdNVP may result in drug resistance mutations (DRM), it is now recommended that HIV-infected infants receive a lopinavir/ritonavir (LPV/r)-based treatment regimen. In a country such as Tanzania, this effectively means starting infants on a second-line therapy in a setting where third-line options are scarce.³ Little data from Tanzania are available to inform whether HIV non-nucleoside reverse transcriptase inhibitor (NNRTI) resistance is present among infected infants under the Option A guidelines. This study aimed to identify primary resistance mutations in HIV-infected infants and to identify risk of NVP resistance based on maternal and infant NVP exposure.

Materials and Methods

This was a retrospective study that analyzed dried blood spot (DBS) cards of HIV-infected infants from two regions of Tanzania (Kilimanjaro and Arusha) collected from January 1, 2013 to May 31, 2014. The Kilimanjaro Christian Medical Centre (KCMC) Clinical Laboratory is the early infant diagnosis (EID) testing laboratory for the northern zone of Tanzania. The laboratory registry book was reviewed for positive DBS polymerase chain reaction (PCR) results. Available DBS cards, which were stored at room temperature in a plastic zippered-lock bag with desiccant per standard protocol, were retrieved and sent to the Duke Human Vaccine Institute (DHVI) for viral load confirmation and resistance testing.

DBS cards typically have five circles (spots) filled with blood. Two were used for initial testing, and some cards had additional spots used for quality assurance testing at KCMC, leaving only one half to three spots available for subsequent use. Participants were excluded if the DBS card had insufficient sample remaining or results between DHVI and KCMC were discordant. If multiple cards were available from a single infant, the participant's earliest positive DBS card was used for the study.

Viral load was determined using the Roche COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, version 2.0. DBS cards were processed according to COBAS® AmpliPrep/COBAS TaqMan HIV-1 Qual Test package insert to free the viral material from the DBS. The resulting DBS/SPEX eluate was assayed on the COBAS AmpliPrep/COBAS TaqMan HIV-1 Test, version 2.0 platform according to manufacturer's instructions. Six microliters of RNA eluate was used for reverse transcription using SuperScript III first-strand synthesis Superscript (Invitrogen, Carlsbad, CA) following manufacturer's instruction, which synthesized 20 µl of cDNA. The first round amplification used 1 µl of cDNA for nested PCR by amplification of the Gag-pol gene with both primer positions relative to HXB2 genome. PCR primers included Gagout primer position 1817 through 1847 (5' TAGAAGAAATGATGAC-AGCATGTCTAGGGAGT 3') and Polout primer position 2069

through 2095 (5'-CTA ACT TCT GTA TAT CAT TGA CAG TCC A-3'). Second round amplification used 2 µl of first round PCR products for second round amplification PCR products using primers Gagin primer position 2069–2095 (5' CTGA-GAGACAGGCTAATTTTTTAGGGA 3') and Polin primer position 3236 through 3210 (5'-CAT CCA AAG GAA TGG AGG TTC TTT CTG ATG -3'). Second round PCR products were preceded by cycle sequencing and dye terminator methods with an ABI 3700 genetic analyzer (Applied Biosystems, Foster City, CA). Sequences generated were edited using the Sequencher program 5.0 (Gene Codes, Ann Arbor, MI). Sequences were aligned and a phylogenetic tree of HIV subtypes was drawn by using SeaView.^{4,5} Sequences were submitted to Stanford HIV Drug Resistance Database for interpretation^{6,7} and accession numbers assigned in GenBank.

Local PMTCT sites with identified positive DBS result from the KCMC Zonal Laboratory were visited and maternal and infant PMTCT data were extracted from the PMTCT registry book. The PMTCT registry book is a handwritten logbook, maintained by trained nursing staff who document prespecified information such as date the woman entered into the registry, status of HIV disclosure to the father of the child, and maternal PMTCT regimen received. Maternal PMTCT regimens were defined in the registry as (1) none, (2) sdNVP, (3) Option A, or (4) ART (regimen not otherwise defined).

The PMTCT registry also contained information about the infant including date of birth, birth weight, gender, receipt of NVP, feeding regimen, and whether NVP was weight adjusted at subsequent visits. The date DBS was collected, the date the DBS PCR result was received, whether or not a referral to HIV care was made were also documented in the registry.

This study defined categories of infant NVP exposure as follows: (1) No NVP exposure included infants with documentation that no NVP was given to the infant and no PMTCT medications were given to the mother; (2) NVP exposure included infants with documentation stating either the infant received NVP and/or maternal PMTCT included either sdNVP or Option A; (3) unknown NVP exposure included infants for whom either PMTCT information was not documented (unknown) regarding maternal PMTCT regimen or infant NVP, or whether the mother received ART, because whether NVP was part of the antiretroviral regimen was not explicitly defined in the registry.

Data were summarized using summary statistics: means and standard deviation (SD) or median and interquartile range (IQR) for continuous variables and by counts and percentages for categorical variables. Demographics are presented comparing DBS card samples that were able to amplify for resistance sequences versus those without resistance amplification. Statistical tests appropriate for the nature of the outcome were used: chi-square for categorical variables or Fisher's exact test when assumptions (i.e., expected counts <5) were not satisfied and Wilcoxon rank sum test for continuous variables. For those with resistance data, mutations were separately summarized according to the maternal regimen and the child regimen, as well as according to known NVP exposure. Statistical analyses were performed using Stata 13.1.

Ethics

The Duke University Institutional Review Board, the KCMC Ethics Committee, and the National Institute of

Medical Research in Tanzania approved the study. A signed material transfer agreement was obtained between the KCMC Clinical Laboratory and DHVI.

Results

A total of 184 infants had a positive DNA PCR. As demonstrated in Figure 1, 18 samples were excluded: 2 because of a discordant result. Among the 166 DBS cards included in the study, 3 did not have numeric viral load results due to the presence of inhibitors. Demographics of infants with and without resistance data are presented in Table 1. The median infant age was 111 days (IQR 47–238) and 56% were female. Average viral load $\log_{10}$ copies/ml was 5.01 (SD 0.77). DBS cards with higher viral load were significantly more likely to be amplified for resistance testing ($p < .001$). Samples with fewer than 5,000 copies/ml did not amplify (11/163). An infant having received NVP as part of PMTCT was also associated with availability of resistance data (49.5% vs. 27.7%, $p = .02$). Otherwise, the two groups were similar.

Of the 65 infants with drug resistance data, NNRTI mutations were present in 34 infants (52%), of which 24/34 (71%) were high-level resistance mutations (HLRMs) to NVP (Table 2). Mutations were not mutually exclusive, many samples

had more than one mutation (Fig. 2). Among infants not exposed to NVP, 1/13 (8%) had a HLRM compared with 14/22 (64%) of the infants known to be exposed to NVP ($p = .002$). Among infants with uncertain NVP exposure, 9/30 (30%) had HLRMs to NVP (Table 2). Of those with NRTI mutations, 9/12 (75%) had one or more thymidine analogue mutations (TAMs). Intermediate-to-high-level resistance to AZT and abacavir (ABC) was present in four samples, each with two or more TAMs present. No clinically significant PI mutations were detected. Multiple viral subtypes were present, with subtype A being the most common (Fig. 3). There was no significant difference in percentage HLRM between subtypes ($p = .98$).

Discussion

Our data demonstrate that infants exposed to NVP as part of PMTCT are at increased risk of high-level resistance to NVP compared with infants who were not exposed to NVP. This is consistent with the reduced rate of viral suppression seen when HIV-infected infants are treated with NVP-based regimens compared with a PI-based regimen. For example, IMPAACT P1060 evaluated treatment outcomes of infants in India and nine sub-Saharan African countries comparing NVP with LPV/r.^{8,9} Infants exposed to NVP prophylaxis and treated with zidovudine, lamivudine, and NVP were more likely to demonstrate virologic failure at 24 weeks (HIV-1 RNA >400 copies/ml or $<1 \log_{10}$ reduction from baseline) than those receiving zidovudine, lamivudine, and lopinavir/r (ritonavir) ($p = .02$).⁸ Baseline NVP resistance mutations were detected in 12% of 148 baseline viral samples obtained from NVP-exposed infants, and mutations were predictive of NVP treatment failure. Our study demonstrated an even higher rate of HLRM, at 37% (24/65). Our findings are similar to a study from neighboring Mozambique, which demonstrated 46% HLRM (36/79) with a 30-fold increased odds of HLRMs among infants whose mother was exposed to Option A prophylaxis as compared with those infants whose mother received ART ($p < .001$). Infant exposure to NVP was not a significant risk for infant NVP resistance in this Mozambican study.¹⁰ A more recent study from Togo using methodology similar to this Tanzanian study found that 60% of newly diagnosed infants had detectable DRMs and 57% with HLRM to NVP.¹¹ Similarly, 57% of newly diagnosed infants exposed to PMTCT in South Africa had NNRTI resistance.¹² Younger infants were more likely to have NNRTI drug resistance in both studies.^{11,12} In Senegal, the proportion of children whose viruses harbored HLRM were 6.5-fold higher in children whose mother–infant pairs had received NVP-based chemoprophylaxis than in pairs without prophylaxis [7 of 13 (53.8%) vs. 1 of 12 (8.3%)].¹³ The majority of NNRTI resistance mutations in these studies were consistently Y181C and K103N. Having additional “minor resistant variants” of NVP resistance mutations may further increase risk of treatment failure,^{14,15} and NVP-resistant virus may persist beyond 1 year.^{16,17}

Cohort 2 of IMPAACT P1060 evaluated treatment outcomes of infants who were not exposed to NVP prophylaxis. Results demonstrated that despite only 2% of baseline viral sequences having HLRM in the non-NVP exposed cohort, these infants also had superior treatment outcomes on LPV/r-based regimens than NVP-based treatment ($p < .001$).⁹ In the Togo study, 41 of 201 (20%) infected infants had no exposure to PMTCT, yet 11/41 (27%) of these infants had HLRM.¹¹ WHO guidelines now recommend that all children under

FIG. 1. Flow diagram of infants with positive DNA PCR from KCMC Northern Zone Laboratory Registry. KCMC, Kilimanjaro Christian Medical Centre; PCR, polymerase chain reaction.

TABLE 1. DEMOGRAPHICS COMPARING SAMPLES WITH AND WITHOUT RESISTANCE DATA

Characteristic: median (IQR) ^a or n (%)	Total N = 166	Resistance data not available n = 101	Resistance data available n = 65	p
Median infant age at time of DBS test in days (IQR)	111 (47–238)	106 (44–259)	112 (54–221)	.98
Gender (female)	93 (56%)	57 (56%)	36 (55%)	.87
Maternal HIV status disclosed to partner	68 (41%)	44 (44%)	24 (37%)	.63
Infant referred to HIV care clinic	56 (34%)	37 (34%)	19 (22%)	.43
Viral load (log), mean (SD) (n = 163)	5.01 (0.77)	4.78 (0.80)	5.37 (0.57)	<.001
Infant feeding				
EBF	87 (52.4%)	57 (56.4%)	30 (46.2%)	.15
RF	27 (16.3%)	14 (13.9%)	13 (20.0%)	
Mixed	8 (4.8%)	7 (6.9%)	1 (1.6%)	
Unknown	44 (26.5%)	23 (22.8%)	21 (32.3%)	
Maternal regimen				
sdNVP	28 (16.9%)	18 (17.8%)	10 (15.4%)	.63
Option A	14 (8.4%)	10 (9.9%)	4 (6.2%)	
ART	30 (18.1%)	20 (19.8%)	10 (15.4%)	
None	39 (23.5%)	24 (2.8%)	15 (23.1%)	
Unknown	55 (33.1%)	29 (28.7%)	26 (40.0%)	
Infant regimen				
NVP given	68 (41.0%)	50 (49.5%)	18 (27.7%)	.02
NVP not given	49 (29.5%)	25 (24.8%)	24 (36.9%)	
NVP unknown	49 (29.5%)	26 (25.8%)	23 (35.4%)	

^aUnless otherwise stated.

EBF, exclusive breastfeeding; IQR, interquartile range; NVP, nevirapine; RF, replacement feeds.

3 years of age initiate ART with an LPV/r-based regimen.³ Tanzanian guidelines adopted this policy in 2015,¹⁸ although implementation has taken time due to higher cost and a limited stock of liquid LPV/r.

Two infants with a positive DBS (qualitative) result from the KCMC Clinical Laboratory did not have a detectable viral load (quantitative RNA) when the testing was performed for this study. These discrepant results were reported back to the PMTCT center where infants were receiving care, with attempts to trace the two infants. One infant had reportedly died, and the second infant had reportedly moved to another

town and was not traceable. Neither infant had documentation of referral for HIV treatment.

Only one other published article has reported resistance mutations using DBS cards among infants in Tanzania. This publication had similar findings regarding subtype distribution and the predominance of Y181C and K103 N mutations, although it did not have concurrent clinical PMTCT registry data regarding NVP exposure.¹⁹ Two studies from Dodoma, Tanzania, report resistance data from different populations including HIV-infected, ART naive pregnant women with 7.5% harboring NNRTI mutations²⁰ and ART naive children

TABLE 2. NNRTI RESISTANCE MUTATIONS EXPECTED TO CONFER RESISTANCE IN 65 HIV-INFECTED INFANTS BY PMTCT REGIMEN

Resistance data	NNRTI resistance mutations	None	Any	HLRM ^a
	Total (N = 65)	31 (48%)	34 (52%)	24 (37%)
Maternal regimen	sdNVP+tail (n = 10)	3 (30%)	7 (70%)	6 (60%)
	Option A (n = 4)	0 (0%)	4 (100%)	4 (100%)
	ART (n = 10)	4 (40%)	6 (60%)	4 (40%)
	None (n = 15)	12 (80%)	3 (20%)	1 (7%)
	Unknown (n = 26)	12 (46%)	14 (54%)	9 (35%)
Infant regimen	NVP given (n = 18)	4 (22%)	14 (78%)	13 (72%)
	NVP not given (n = 24)	15 (62%)	9 (38%)	3 (13%)
	NVP (unknown) (n = 23)	12 (52%)	11 (48%)	8 (35%)
NVP exposure	Known NVP exposure (n = 22)	6 (27%)	16 (73%)	14 (64%)
	Known to not be exposed to NVP (n = 13)	11 (85%)	2 (15%)	1 (8%)
	Unknown exposure to NVP (n = 30)	14 (47%)	16 (53%)	9 (30%)

Bold text meets criteria for known nevirapine exposure.

NNRTI high-level resistance mutations include Y188L, K103 N/S, Y181C, V106A, and G190A.

^aSubset of any.

ART, antiretroviral therapy; HLRM, high-level resistance mutation; NNRTI, non-nucleoside reverse transcriptase inhibitor; sdNVP, single dose nevirapine; tail, zidovudine+lamivudine for 1 week after sdNVP.

FIG. 2. Non-nucleoside reverse transcriptase mutations are present in over half (34/65) of HIV sequences. Mutations are not mutually exclusive.

(mean age 8 years) with 69.6% demonstrating NNRTI mutations among presumed ART naive children.²¹

The average age of infants at the time of first positive DBS was nearly 3 months (111 days) with a broad IQR (47–238 days). Tanzanian guidelines recommend initial DBS testing at 4–6 weeks of life. UNICEF data report that only half of HIV-exposed babies are tested for HIV by the age of 2 months.¹ This delayed age to first positive DBS for HIV-infected infants is likely multifactorial, reflecting broken linkages within the PMTCT cascade, reluctance to test due to stigma, or delay in infant testing until the infant shows signs of illness.

Only one-third of HIV-infected infants in this study had a documented referral to care. This is a very concerning finding contributing to the leaky PMTCT cascade and disruption in the continuum of care. Use of mobile clinics is not only for convenience but also often anonymity. Positive DBS results from the clinical laboratory were frequently not recorded in the main PMTCT registry book, but rather documented at the mobile clinics that were often unavailable for review. Mobile clinics may be less likely to provide results and trace positive infants in the community. Less than half of women reported disclosure of maternal HIV status to the father of the child (41%), likely due to stigma and fear of abandonment. Community stigma certainly contributed to difficulty tracing and referring HIV-infected infants to HIV care.

There were several limitations to the study, including missing clinical data from incompletely filled PMTCT registry books. Over one-third of mother–infant pairs had an unknown PMTCT regimen, though they presumably had NVP exposure as NVP was standard for PMTCT prophylaxis and ART for pregnant women during the timeframe of the study; 30% of sequenced virus in this unknown NVP exposure group had HLRM. Information regarding whether a woman had prior exposure to PMTCT from a prior pregnancy was not available, thus infants categorized as no NVP exposure are based solely on the maternal exposure during the reported pregnancy. Maternal CD4 results at the time of delivery were not available and HIV viral load testing was not the standard of care at the time of this study.

Another limitation was that less than half (39%) of DBS cards could be amplified for resistance testing. Low sequencing rates were multifactorial. A study from Dodoma, Tanzania, reported a DBS amplification and sequencing rate of 44% compared with 95% from plasma samples in the same study.²¹ Second, low levels of virus may have impacted sequencing success as samples with viral load <5,000 copies/ml (7%) were not able to be sequenced. The World Health Organization recommends programs relying on the use of DBS to maintain a higher threshold of 3,000–5,000 copies/ml of positivity until the sensitivity at lower thresholds has been

FIG. 3. Viral subtypes and percentage of each subtype containing high-level resistance mutations. Mutations are present in all subtypes. Recombinant subtypes include A/C (4.7%), A/D (4.7%), AG (1.6%), and UD (1.6%).

confirmed.²² A previous study from the KCMC site in Tanzania compared plasma RNA with DBS for EID and estimated high DBS sensitivity and specificity at the threshold of $\geq 1,000$ copies/ml, though performance based on a linearity plot was best for samples $> 5,000$ copies/ml.²³ A systematic review of 19 studies that compared virologic results from different blood collection methods (plasma or whole blood versus DBS) suggested that lower rates of viral load detection (< 40 copies/ml) in plasma than in DBS may be due to the low input volume in DBS (20–25 μ l) compared with 100 μ l–1.0 ml in plasma.²⁴ Low input volume was certainly a challenge in this study with several DBS cards having only 1/2 to 1 spot remaining on the DBS card.

Storage conditions in this study were at ambient temperature and did not aim for future genetic testing. This may have resulted in viral degradation over time. An evaluation by the Global World Health Organization Laboratory Network for quality assurance suggested that DBS cards shipped on dry ice had results comparable with samples shipped at ambient temperature, though DBS cards were prepared under ideal conditions using pipetted blood in a controlled laboratory setting as opposed to an infant heel prick in the field. Samples were then stored at -80°C upon arrival at the respective laboratory before sequence testing.²⁵ Another multicountry comparison of plasma and DBS viral load and genotype testing found 77% genotyping was successful using DBS stored at ambient temperature for 2–4 weeks and subsequently stored at -20°C .²⁶ From rural Tanzania, samples stored at ambient temperature for up to 3 months and sequenced in-house were successfully genotyped, 34/36 (96%), without a cold chain.²⁷ In this study, however, samples remained for > 1 year stored and shipped at ambient temperature. The methodology and size of the punch from filter paper spots, the elution buffer and extraction method, and sample volumes after extraction may all affect the ability to amplify and sequence virus.^{24,28}

Conclusions

Clinically significant NNRTI mutations are common among HIV-infected infants in Tanzania. LPV/r-based ART initiation in HIV-infected infants is recommended and supported by these research findings, although using “second-line” therapy as first-line may leave fewer ART options later in life. Additional attention is needed in the infant treatment cascade. Clinical site data showed low rates of referral for infected infants and incompletely filled registry books. As Option B+ is scaled up, re-evaluation of the preferred infant starting regimen will be necessary.

GenBank

Accession numbers are KY747326 through KY747392. Please note KY747334 was excluded as this sample is from outside the defined catchment region; KY747349 was excluded as it is a repeat sample from the same participant whereby KY747346 was the initial DBS positive sample.

Acknowledgments

The authors would like to sincerely thank our research assistant Tumsifu Tarimo for his efforts in collecting clinical data in the field; Feng Gao and Thomas Denny for their su-

pervision at Duke Human Vaccine Institute, Duke Medical Center, Durham, NC; the KCMC Clinical Laboratory staff; the PMTCT site clinical staff; and the registered mother–infant pairs for their help in making this study possible. This research was supported by the President’s Emergency Plan for AIDS Relief (PEPFAR) through a supplement to the National Institute of Allergy and Infectious Diseases (NIAID): NOT-AI-10-023 (3U01 AI069484 A4S2); the International Research Scientist Development Award (K01 TW-009985) funded by the Fogarty International Center and the National Institute of Mental Health; and Duke Center for AIDS Research (5P30 AI064518). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the article.

Author Disclosure Statement

No competing financial interests exist.

References

1. UNICEF: Situation: Children, Adolescents, and AIDS in 2016. Available at <http://childrenandaids.org/situation>, accessed January 23, 2017.
2. WHO: Consolidated Guidelines on the use of Antiretroviral Drugs for Treating and Preventing HIV Infection. Recommendations for a public health approach-second edition (page 100). Available at www.who.int/hiv/pub/guidelines/arv2013/download/en (2013), accessed December 2, 2016.
3. WHO: Consolidated Guidelines on the use of Antiretroviral Drugs for Treating and Preventing HIV Infection. Recommendations for a public health approach-second edition (page 122). Available at www.who.int/hiv/pub/guidelines/arv2013/download/en (2013), accessed December 2, 2016.
4. Gouy M, Guindon S, Gascuel O: SeaView version 4: A multiplatform graphical user interface for sequence alignment and phylogenetic tree building. *Mol Biol Evol* 2010;27:221–224.
5. PRABI-Doua (Pole Rhone-Alpes de Bioinformatique Site). Available at <http://pbil.univ-lyon1.fr/software/seaview>, accessed December 5, 2015.
6. Liu TF, Shafer RW: Web resources for HIV type 1 genotypic-resistance test interpretation. *Clin Infect Dis* 2006; 42:1608–1618.
7. Stanford University HIV Drug Resistance Database. Available at <http://sierra2.stanford.edu/sierra/servlet/JSierra>, accessed December 5, 2016.
8. Palumbo P, Lindsey JC, Hughes MD, *et al.*: Antiretroviral treatment for children with peripartum nevirapine exposure. *N Engl J Med* 2010;363:1510–1520.
9. Violar A, Lindsey JC, Hughes MD, *et al.*: Nevirapine versus ritonavir-boosted lopinavir for HIV-infected children. *N Engl J Med* 2012;366:2380–2389.
10. Antunes F, Zindoga P, Gomes P, *et al.*: Development of Nevirapine Resistance in Children Exposed to the Prevention of Mother-to-Child HIV-1 Transmission Programme in Maputo, Mozambique. *PLoS One* 2015;10:e0131994.
11. Salou M, Butel C, Konou AA, *et al.*: High rates of drug resistance among newly diagnosed HIV-infected Children in the National Prevention of Mother-to-child Transmission Program in Togo. *Pediatr Infect Dis J* 2016;35:879–885.
12. Kuhn L, Hunt G, Technau KG, *et al.*: Drug resistance among newly diagnosed HIV-infected children in the era of more efficacious antiretroviral prophylaxis. *AIDS* 2014;28:1673–1678.

13. Kebe K, Belec L, Diop Ndiaye H, *et al.*: The case for addressing primary resistance mutations to non-nucleoside reverse transcriptase inhibitors to treat children born from mothers living with HIV in sub-Saharan Africa. *J Int AIDS Soc* 2014;17:18526.
14. Lehman DA, Wamalwa DC, McCoy CO, *et al.*: Low-frequency nevirapine resistance at multiple sites may predict treatment failure in infants on nevirapine-based treatment. *J Acquir Immune Defic Syndr* 2012;60:225–233.
15. MacLeod IJ, Rowley CF, Thior I, *et al.*: Minor resistant variants in nevirapine-exposed infants may predict virologic failure on nevirapine-containing ART. *J Clin Virol* 2010;48:162–167.
16. Martinson NA, Morris L, Gray G, *et al.*: Selection and persistence of viral resistance in HIV-infected children after exposure to single-dose nevirapine. *J Acquir Immune Defic Syndr* 2007;44:148–153.
17. Kanthula R, Beck I, VanDyk G, Silverman R, Olson S, Salyer C, Cassol S, Rossouw T, Frenkel L: Persistence of HIV Drug Resistance in Children Exposed to Nevirapine Prophylaxis. Conference on Retroviruses and Opportunistic Infections (CROI). Abstract # 847. Boston, MA, February 22–25, 2016.
18. The United Republic of Tanzania Ministry of Health and Social Welfare: National Guidelines for the Management of HIV and AIDS. National AIDS Control Programme (NACP) Fifth edition, Ministry of Health and Social Welfare National AIDS Control Program, Dar es Salaam. May 2015. pp. 149–150.
19. Shao ER, Kifaro EG, Chilumba IB, *et al.*: HIV-1 drug mutations in children from northern Tanzania. *J Antimicrob Chemother* 2014;69:1928–1932.
20. Vairo F, Nicastrì E, Liuzzi G, *et al.*: HIV-1 drug resistance in recently HIV-infected pregnant mother's naïve to antiretroviral therapy in Dodoma urban, Tanzania. *BMC Infect Dis* 2013;13:439.
21. Meini G, Balestrieri M, Cianchino S, *et al.*: Frequent detection of antiretroviral drug resistance in HIV-1-infected orphaned children followed at a donor-funded rural pediatric clinic in Dodoma, Tanzania. *AIDS Res Hum Retroviruses* 2015;31:448–451.
22. World Health Organization: Consolidated Guidelines on the Use of Antiretroviral Drugs for Treating and Preventing HIV Infection. June 2013, p 136. Available at www.who.int/hiv/pub/arv/arv-2016/en, accessed June 10, 2017.
23. Lofgren SM, Morrissey AB, Chevallier CC, *et al.*: Evaluation of a dried blood spot HIV-1 RNA program for early infant diagnosis and viral load monitoring at rural and remote healthcare facilities. *AIDS* 2009;23:2459–2466.
24. Smit PW, Sollis KA, Fiscus S, *et al.*: Systematic review of the use of dried blood spots for monitoring HIV viral load and for early infant diagnosis. *PLoS One* 2014;9:e86461.
25. Parkin N, de Mendoza C, Schuurman R, *et al.*: Evaluation of in-house genotyping assay performance using dried blood spot specimens in the Global World Health Organization laboratory network. *Clin Infect Dis* 2012;54 Suppl 4:S273–S279.
26. Monleau M, Aghokeng AF, Eymard-Duvernay S, *et al.*: Field evaluation of dried blood spots for routine HIV-1 viral load and drug resistance monitoring in patients receiving antiretroviral therapy in Africa and Asia. *J Clin Microbiol* 2014;52:578–586.
27. Johannessen A, Holberg-Petersen M, Lovgaarden G, *et al.*: HIV type-1 drug resistance testing on dried blood spots is feasible and reliable in patients who fail antiretroviral therapy in rural Tanzania. *Antivir Ther* 2010;15:1003–1009.
28. Monleau M, Montavon C, Laurent C, *et al.*: Evaluation of different RNA extraction methods and storage conditions of dried plasma or blood spots for human immunodeficiency virus type 1 RNA quantification and PCR amplification for drug resistance testing. *J Clin Microbiol* 2009;47:1107–1118.

Address correspondence to:

Dorothy E. Dow

Division of Infectious Diseases

Department of Pediatrics

Duke University Medical Center

DUMC Box 3499

Durham, NC 27710

E-mail: dorothy.dow@duke.edu