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Sigaloff, K.C.E.

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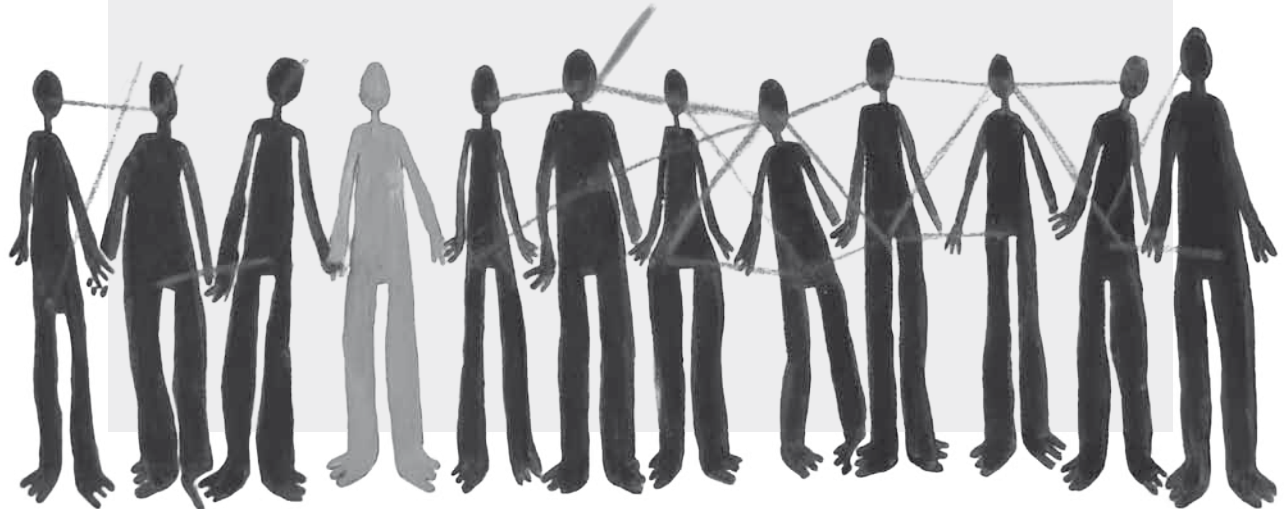
Chapter 12

High rates of thymidine analogue mutations and dual-class resistance among HIV-infected Ugandan children failing first-line antiretroviral therapy

Kim C.E. Sigaloff, Joshua Kayiwa, Victor Musiime, Job C.J. Calis, Elizabeth Kaudha, Andrew Mukuye, Christine Matama, Immaculate Nankya, Lillian Nakatudde, John T. Dekker, Raph L. Hamers, Peter Mugyenyi, Tobias F. Rinke de Wit, Cissy Kityo

Submitted



ABSTRACT

Background

HIV-infected children are at high risk of acquiring drug resistant viruses, which is of particular concern in settings where antiretroviral drug options are limited. We aimed to assess resistance patterns and predict viral drug susceptibility among children with first-line antiretroviral therapy (ART) failure in Uganda. A cross-sectional analysis of children switching ART regimens due to first-line failure was performed at three clinical sites in Uganda.

Methods

HIV-RNA determination and genotypic resistance testing on all specimens with HIV-RNA >1000 copies/ml was performed. Major drug resistance mutations were scored using the 2011 International Antiviral Society–USA list. The Stanford algorithm was used to predict drug susceptibility.

Results

At the time of switch, 44 genotypic resistance tests were available for 50 children. Viruses with dual-class resistance in the nucleoside (NRTI) and non-nucleoside reverse transcriptase inhibitor (NNRTI) drug-class were isolated in 98% (95% confidence interval [CI] 88-100%). Forty-six percent (95% CI 30-61%) of children harbored multiple thymidine analogue mutations. M184V was identified as the only NRTI mutation in 27% (95% CI 15-43%). HIV susceptibility to NRTIs, with the exception of tenofovir, was reduced in ≥60% of children.

Conclusion

Ugandan children experiencing first-line ART failure in our study harbored high rates of dual-class and accumulated HIV drug resistance. Methods to prevent treatment failure, including adequate pediatric formulations and alternative second-line treatment options are urgently needed.

INTRODUCTION

An estimated 3.4 million children are currently infected with HIV-1 worldwide, of whom 90% reside in sub-Saharan Africa.[1] Antiretroviral therapy (ART) has strikingly reduced morbidity and mortality in children.[2] However, younger children typically have poorer virological responses than adults,[3-5] which is attributable to high viral loads before treatment,[6] and the risk of sub-therapeutic drug concentrations owing to limited pediatric formulations, variable pharmacokinetics and changes in bodyweight.[7] Suboptimum virological suppression fosters the emergence of drug resistant viruses, the consequences of which are particularly important for children as they require ART for longer periods of time than adults.[8]

In resource-limited settings, exposure to nevirapine for the prevention of mother-to-child transmission (PMTCT) increases the risk of HIV drug resistance development and subsequent treatment failure of standard first-line regimens containing non-nucleoside reverse transcriptase inhibitors (NNRTIs).[9, 10] Due to financial and technical constraints, virological monitoring to enable early failure detection is often not available. Furthermore, the identification of resistance mutations using genotypic testing, which could aid appropriate sequencing of antiretroviral medication, is seldom performed. As a consequence, children in resource-limited settings are empirically switched to a second-line regimen, typically containing ritonavir-boosted lopinavir and two new and/or recycled nucleoside reverse transcriptase inhibitors (NRTIs).

In Uganda, an estimated 200,000 children are HIV-infected and the Joint Clinical Research Centre (JCRC) is an important provider of HIV care and treatment in Uganda. In this cross-sectional study, we describe the resistance patterns among children failing first-line ART at three JCRC clinical sites, and predict viral drug susceptibility in order to gain insight into the optimal strategies for second-line therapy.

MATERIALS AND METHODS

Study design and population

The Monitoring Antiretroviral Resistance in Children (MARCH) study is a multicenter prospective observational cohort of HIV-1 infected children who receive ART at 3 clinical sites in Uganda. The national JCRC network – consisting of 7 clinical sites – has over 20 years of experience with ART, from conducting clinical trials to nationwide roll-out programs since 2003. Site and cohort characteristics have been described previously.[11]

Potential participants were informed of the study and screened for eligiblity by the study staff. Children aged 12 years or below, that initiated a first- or second-line ART regimen were enrolled. For the current analysis, only children requiring a treatment switch from first- to second-line ART due to treatment failure were included. Local definitions of clinical, immunogical and virological treatment failure were in accordance with WHO guidelines, i.e. virological failure was defined as a persistent viral load >5000 copies/ml after at least 6 months of treatment.[12] The ethical committees of JCRC in Uganda and the Academic Medical Center of the University of Amsterdam in The Netherlands approved the study protocol. The parent(s)/guardian(s) of all eligible children provided written informed consent. Children above the age of eight who were aware of their HIV status provided written informed assent. Socio-demographic and clinical data were collected using electronic case report forms, which were aggregated in a web-based database.

Procedures

Routine laboratory results including CD4 cell count and serum hemoglobin concentration were obtained from local laboratory records. Immunodeficiency for age was classified according the 2010 WHO guidelines.[12] HIV-RNA and genotypic resistance testing on all specimens with HIV-RNA >1000 copies/ml was performed at a central reference laboratory. For HIV-RNA determination, the COBAS Ampliprep/COBAS Taqman HIV-1 test (Roche, Branchburg, NJ, USA) was used. For HIV-1 genotyping, an in-house sequencing method encompassing the whole of PR and codons 1-300 of RT with a Beckman Coulter CEQ 8000 analyzer (Beckman Coulter Inc., Fullerton, CA, USA) was used.[13] Sequences were assembled and manually edited using BioEdit software version 7.1.3.0. Subtypes were determined using MEGA 5.10 software. Drug resistance mutations were scored according to the 2011 International AIDS Society-USA list.[14] HIV drug susceptibility for each participant was predicted using the Stanford algorithm, version 6.1.0[15] and was categorized as susceptible, potential low-level resistance, low-level resistance, intermediate resistance, or high-level resistance. The proportions of virus with drug resistance mutations were calculated with 95% confidence intervals (CI). Analyses were performed using Stata version 10 (StataCorp LP, TX, USA).

RESULTS

Between January 2010 and July 2011, 50 children who switched to second-line after first-line ART failure were enrolled. The patients originated from Kampala (n=29), Mbale (n=18) and Fort Portal (n=3). The median age was 5.5 years old (interquartile range [IQR] 4.0-10.1) and 50% (25/50) of the participants were girls (Table 1). The mother was the primary caregiver for most children (n=30, 60%); 9 (18%) children were orphans. At the

Table 1. Characteristics of children with first-line antiretroviral therapy failure

	Total (n=50)
Age (years)	5.5 (4.0-10.1)
Sex, female	25 (50.0)
Living situation	
With 2 parents	13 (26.0)
With 1 parent	24 (48.0)
Other	13 (26.0)
Mother current ART	
First-line ART	23 (46.0)
Second-line ART	3 (6.0)
Not on ART	5 (10.0)
Mother deceased	14 (28.0)
Unknown	5 (10.0)
WHO clinical stage	
1 or 2	27 (54.0)
3 or 4	23 (46.0)
Weight-for-age z-score[a]	
Underweight (< -2 SD)	9 (24.3)
Height-for-age z-score	
Stunting (< -2 SD)	25 (50.0)
CD4 % (in <5 year)	23.2 (14.0-30.0)
CD4 cell count (in ≥5 year)	460.5 (86.0-645.5)
Total lymphocyte count (x 10^9/L)[b]	3.0 (1.8-3.9)
Hemoglobin (g/dl)[c]	11.3 (10.3-12.7)
Viral load (log_{10})	4.8 (4.0-5.2)
PMTCT exposure	
Yes	13 (26.0)
No	37 (74.0)
Previous ART regimen	
AZT/3TC/NVP or EFV	35 (70.0)
D4T/3TC/NVP	10 (20.0)
ABC/3TC/NVP or EFV	4 (8.0)
AZT/3TC/ABC	1 (2.0)
Duration of first-line ART (months)[d]	29.8 (25.1-48.4)

Data presented as n (%) or median (interquartile range). ART = antiretroviral therapy, SD = standard deviation, PMTCT = prevention of mother-to-child transmission, AZT = zidovudine, 3TC = lamivudine, NVP = nevirapine, EFV = efavirenz, D4T = stavudine, ABC = abacavir. [a]Weight-for-age z-score calculated for ages 0 to 10 year only, n=37; [b]Total lymphocyte count, n=48; [c]Hemoglobin, n=48; [d]Duration of first-line ART, n=49.

Table 2. Mutational patterns among children with first-line antiretroviral therapy failure and available genotypic test results

Resistance pattern	Overall (n=44)
Any mutation	44 (100) [92-100]
Any NRTI	43 (97.7) [88-100]
Any TAM	28 (63.6) [28-78]
≥ 2 TAMs	20 (45.5) [30-61]
≥ 3 TAMs	16 (36.4) [22-52]
TAM+M184V	28 (63.6) [28-78]
M184V only	12 (27.3) [15-43]
Any NNRTI	44 (100) [92-100]
≥ 2 NNRTIs	24 (54.5) [39-70]
NRTI+NNRTI	43 (97.7) [88-100]
TAM+NNRTI	28 (63.6) [28-78]
M184V+NNRTI	41 (93.2) [81-99
TAM+M184V+NNRTI	28 (63.6) [28-78]

Data are presented as n (%) [95% confidence interval]. NRTI = nucleoside reverse transcriptase inhibitor, TAM = thymidine analogue mutation, NNRTI = non-nucleoside reverse transcriptase inhibitor.

time of the child's enrolment, 26 (52%) mothers used ART. The decision to switch to second-line ART was based on virological failure in 48 (96%) and immunological failure in 2 (4%). Overall, 23 (46%) children had a WHO stage 3 or 4 event, 9 (24%) children were underweight and 25 (50%) were stunted. Of the children with virological failure, 24 (50%) were also classified as immune-deficient based on their CD4 percentage or CD4 cell count for age.

First-line ART had been received for a median of 30 months (IQR 25.1-48.4). The most common previous regimen consisted of zidovudine/lamivudine with either nevirapine (n=23, 46%) or efavirenz (n=12, 24%), followed by stavudine/lamivudine/nevirapine (n=10, 20%) and abacavir/lamivudine with nevirapine (n=3, 6%) or efavirenz (n=1, 2%). Single-drug substitutions within NRTI drug class were common (n= 23, 46%). Thirteen (26%) children had been exposed to nevirapine for PMTCT (Table 1).

Genotypic tests results were obtained for 44 (88%) children: 4 specimens had a plasma viral load below the sequencing threshold (1000 copies/ml) and 2 specimens failed amplification. Subtype D was most commonly identified (n=19, 43%), followed by A (n=16, 36%), C (n=1, 2%) and various circulating recombinant forms (n=8, 18%). Drug resistance mutations were detected in all children with a genotypic test result (Table 2). Within the NRTI drug class, M184V was present in 41 (93%) and multiple thymidine analogue mutation (≥ 2 TAMs) in 20 (46%). In 64% of children M184V occurred in combination with

≥ 1 TAM and in 27% the M184V was the only NRTI mutation identified. The most frequent NNRTI mutations were K103N (n=17, 39%) and G190A (n=16, 36%). Dual-class NRTI and NNRTI resistance was present in 43 (98%) children (Table 2). Figure 1 depicts frequencies of individuals mutations by the type of NRTI or NNRTI used in the (most recent) first-line regimen. TAMs appeared more frequently when zidovudine was used, whereas K65R was less common (Figure 1A). Multiple NNRTI mutations were observed more frequently when efavirenz was used as opposed to nevirapine (Figure 1B).

Predicted HIV susceptibility to lamivudine and emtricitabine was reduced in nearly all (98%) children due to the high frequency of M184V (Figure 2). For the other NRTIs, reduced susceptibility was predicted for abacavir in 71% of sequences, zidovudine in 59%, stavudine in 68%, didanosine in 61%, and tenofovir in 39%. For the NNRTIs, reduced HIV susceptibility was frequently predicted for efavirenz and nevirapine (98%) and less frequently for the second-generation drugs etravirine and rilpivirine (52%). Reduced susceptibility was not predicted for any of the ritonavir-boosted protease inhibitors (PIs).

DISCUSSION

This cross-sectional study among Ugandan HIV-infected children examined mutational resistance patterns after first-line ART failure and predicted the impact on second-line drug susceptibility. Nearly all children harbored dual-class resistance and multiple TAMs were detected in 46%. HIV susceptibility to NRTIs, with the exception of tenofovir, was severely reduced, jeopardizing the recycling of NRTIs in second-line ART. This issue is of particular concern in young children, for whom the number of licensed NRTIs is limited.

The most frequently observed mutation in our study, the M184V, associated with lamivudine and emtricitabine, delays resistance to zidovudine and stavudine.[16] Despite this increased susceptibility, 64% of patients harbored TAMs in combination with the M184V mutation. The rate of TAMs detected in our study is very high compared to previous reports among children.[8] This may be attributed to the fact that the majority of children were referred from outside clinics and had a longer duration of treatment failure. It also likely reflects clinicians' reluctance to switch to second-line ART, due to regimen cost and subsequent loss of drug options.

After failure of an NNRTI-based regimen in children, the WHO recommends a ritonavir-boosted PI, with a dual backbone of 2 new or recycled NRTIs, i.e. abacavir or zidovudine plus lamivudine.[12] Didanosine is proposed as a possible alternative to lamivudine. In our cohort, susceptibility to abacavir, zidovudine and didanosine was preserved in 29%,

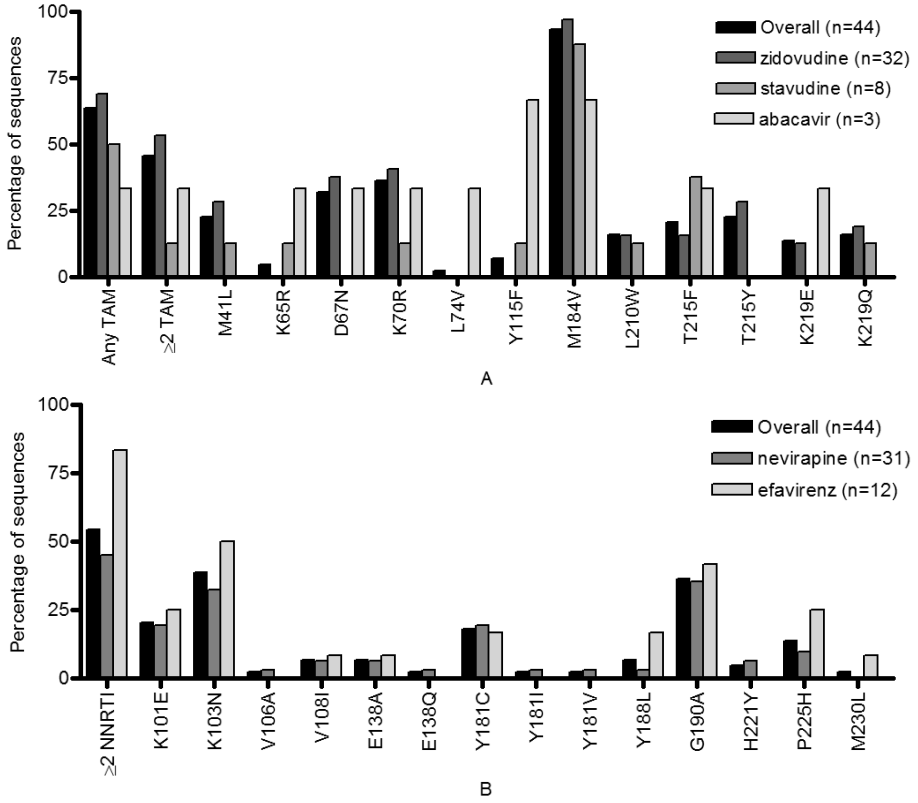


Figure 1. Frequencies of individual drug resistance mutations by nucleoside (A) and non-nucleoside reverse transcriptase inhibitors (B). The legend includes the number of patients exposed to each antiretroviral drug. TAM = thymidine analogue mutation.

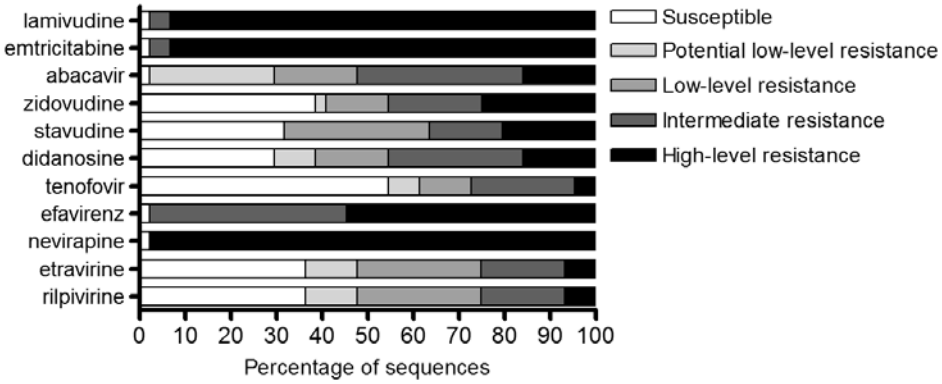


Figure 2. Predicted HIV susceptibility to nucleoside and non-nucleoside reverse transcriptase inhibitors in children with first-line antiretroviral therapy failure (n=44). Genotypic drug susceptibility was predicted using the Stanford HIVdb algorithm (version 6.1.0).

41% and 39% of children, respectively. Therefore, the recommended NRTI backbone in second-line ART would be expected to have very limited effect, while at the same time exposing children to considerable toxicity.[17] The most suitable NRTI to include in second-line ART would be tenofovir, which retains potency despite multiple TAMs. This drug has been associated with promising clinical outcomes in studies,[18, 19] and its recent approval by the US Food and drug Administration (FDA) for children ≥ 2 years of age is particularly encouraging.[20] However, the risk of toxic effects to the kidneys and bone[18] will restrict its use in resource-poor settings, where monitoring is often limited. Therefore, constructing an active NRTI backbone for the children in this study becomes a major challenge.

An alternative strategy for second-line would be the use of boosted-PI monotherapy, which has shown promising preliminary results in adults.[21] In children, this strategy would have several advantages such as fewer pills, fewer drug interactions and less NRTI-associated toxicity. Future research should investigate long-term outcomes of PI monotherapy in the pediatric population. In our study, we profiled the HIV susceptibility to second generation NNRTIs. Susceptibility to etravirine and rilpivirine is reduced in the presence of G190A and Y181C[22] and these mutations were present in 36% and 18% of children, respectively. Etravirine has shown promising results in children,[23, 24] but is not yet licensed for use. For rilpivirine, no data in the pediatric population are yet available.[25] Clearly, the use of newer agents, such as integrase inhibitors, should be explored.

A limitation of our study is the small sample size. However, as resistance data on children is scarce, we believe that the results of this small cohort are important in informing clinicians in resource-limited settings when they are faced with the challenge of changing ART regimens without the aid of genotypic resistance testing. Second, a limitation related to the cross-sectional design is that the prevalent cases identified in this study may not be representative of all incident cases with virological failure.

This study reveals a high proportion of children with multiple TAMs and dual-class resistance after first-line ART failure. As a result, second-line regimens will primarily offer the benefit of the boosted-PI, and limited or no additional effect of the NRTI backbone. Deferred treatment switch is common in resource-limited settings[26, 27] and results in increased mortality.[28, 29] Therefore, the obstacles to second-line drug access and usage must be addressed by reducing costs and increasing the availability of new-generation antiretroviral medications with non-overlapping resistance profiles and tolerable formulations for children.

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