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Resistance in Pediatric Patients Experiencing Virologic Failure with First- and Second-Line Antiretroviral Therapy

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Abstract

We examined HIV-1 resistance in children failing first-and second-line antiretroviral therapy (ART) in South Africa, all with clade C virus. Those exposed to full-dose ritonavir had multiple protease resistance mutations. Nineteen percent had wild type virus. Appropriate ART sequencing in sub-Saharan African children is essential for prolong treatment options.

Keywords

pediatric; antiretroviral therapy; genotype; resource-limited setting; virologic failure; resistance

Antiretroviral therapy (ART) guidelines use conservative approaches to changing therapies due to limited treatment options and high costs. Two ART regimens are available in most resource poor settings: a non-nucleoside reverse transcriptase inhibitor (NNRTI)-based regimen and a boosted protease inhibitor (PI)-based regimen [1,2]. Children require lifelong ART thus necessitating evidence-based approaches to ART selection.

HIV-1 infected children have unique risks for failure and acquisition of drug resistance. The timing of HIV acquisition, along with a developing immune system influences virologic control and resistance development [3]. In South Africa, acceptance of non-suppressed viral loads in the pediatric population on treatment [1] as well as possible exposure to nevirapine

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(NVP) or zidovudine (AZT) monotherapy during prevention of mother to child transmission (pMTCT) programs may increase risk of resistance. Additionally, full-dose ritonavir with two nucleoside reverse transcriptase inhibitors (NRTIs) was routinely prescribed to children <6 months of age for the first 3 years of the South African roll-out program (2004–2007), as lopinavir/ritonavir (LPV/r) was unavailable for these children, increasing the risk for acquisition of PI resistance [1,4].

This is one of the first studies from sub-Saharan Africa to examine drug resistance in children failing first and second-line ART. In this cohort we assessed the use of genotype testing to inform rational sequencing of limited ART options in childhood virologic failure.

METHODS

Setting

The Hannan Crusaid Treatment Centre is a public-sector ART clinic in Cape Town, South Africa. Demographic, immunologic and virologic data has been routinely collected since 2002.

Children received ART according to the South African National ART guidelines [11]. Initial regimen was NNRTI-based or PI-based if the child had been exposed to NNRTIs through pMTCT. LPV/r was the only PI regimen available, except from 2004 to 2007 when children aged <6 months received full-dose ritonavir. Efavirenz (EFV) was prescribed to children ≥3 years and NVP to those <3 years. NRTIs available included AZT, stavudine (d4T), lamivudine (3TC), abacavir (ABC) and didanosine (ddI).

HIV-1 RNA levels (Bayer HIV-1 RNA 3.0 branch DNA assay) and CD4 cell counts were completed at a single local laboratory prior to ART initiation and four-monthly thereafter. Excess serum was stored off-site at Toga Laboratories (Johannesburg, South Africa) at –70 degrees Celsius.

Study population and design

All children less than 16 years old who commenced ART between 2003 and December 2010 were included. This was a retrospective cohort study. Genotypic resistance testing was performed on stored serum samples after any ART failure. Demographic and laboratory data were extracted from the HCTC patient database. Virologic failure was defined as two consecutive viral loads >1000 copies/ml.

Viral sequencing from plasma

Viral RNA was extracted (QIAamp viral RNA minikit, QIAGEN Inc) and HIV-1 protease (PR codons 1-99, HXB2 nucleotides 2254-2549) and reverse transcriptase (RT codons 1-343, HXB2 nucleotides 2550-3577) amplified by a 1-step reverse transcription–polymerase chain reaction (PCR), followed by nested-PCR using gene-specific primers. We report mutations included in the IAS-USA listing [5]. The Stanford University HIV Resistance Interpretation algorithm was used to predict phenotypic resistance patterns from genotype results [6].

Statistical analysis

Baseline, demographic and genotype data were described using medians (with inter-quartile ranges) for numerical data or proportions for categorical data. Genotype data were presented as proportions.

Ethics

The University of Cape Town Research Ethics Committee (REC) approved collection of clinical and genotype data. We obtained written informed consent from every child's caregiver.

RESULTS

Demographic and clinical characteristics

Seventy-one of 472 (15%) children on first-line ART and 15 of 78 (20%) on second-line ART experienced virologic failure by December 2010. The median age of those failing first-line ART was 3.4 years (IQR 1.6–7.8 years) and second-line ART 3.4 years (IQR 2.8–9.0). Twenty-five (35%) failing first-line and nine (60%) failing second-line ART were females. All virus was clade C.

At first-line ART initiation, the median HIV-RNA was 4.8 (IQR 4.5–5.4) $\log_{10}$ copies/ml and CD4 cell count 225 cells/uL (126–457 cells/uL). Median time to first-line ART failure was 2.4 years (IQR, 1.4–3.3 years), and median viral load at failure was 4.0 (IQR 3.5–4.7) $\log_{10}$ copies/ml.

For children commencing second-line median HIV RNA was 3.6 (IQR, 2.9–4.6) $\log_{10}$ copies/ml. Median time to second-line failure was 0.84 years (IQR, 0.55–1.63 years), and median viral load at failure was 3.9 (IQR 3.5–4.3) $\log_{10}$ copies/ml.

First-line failures - genotype data

Thirty-seven (52%) of the 71 children failing first-line ART had stored plasma samples available for genotype testing. Most children were taking NNRTI-based treatment regimens (n=24, 65%), nine (24%) were taking LPV/r-based ART; and four (11%) ritonavir-based treatment. Backbone therapy was d4T or AZT with 3TC. Five children (14%) had been exposed to short-course AZT with single dose NVP in labor as pMTCT, and 1 (2.7%) to NVP alone.

The M184V mutation was present in 22 (59%) of children and thymidine analogue mutations (TAMs) in 7 (19%) children. Virus carrying the K65R mutation was rare (2.7%). NNRTI resistance mutations were present in 24 (65%) children. The most prevalent NNRTI resistance mutations were K103N and V106M. Three of 13 (23%) children failing PI-based first-line therapy also showed the K103N mutation; one exposed to pMTCT, NVP and AZT, and two with unknown pMTCT. Two of 13 (15%) on PI-based regimens had significant protease resistance (PR) mutations (M46I, I54L, L/V82A/F, L90M), both after failing full-dose ritonavir-based therapy. ART regimens and corresponding mutations are displayed in Table 1a.

First-line failures - predicted phenotypic susceptibility

Seven (19%) of the 37 children failed with no significant resistance to any drug group. At first-line ART failure, virus was persistently susceptible to the majority of antiretrovirals, with the exception of 3TC and NNRTIs (see Table A, Supplemental Digital Content 1, <http://links.lww.com/INF/B441>). The proportion with resistance to NNRTIs was similar to the proportion on NNRTI-based regimens. Two children had resistance to LPV/r and other PIs: both had received full-dose ritonavir. Children treated with LPV/r in first-line ART had no major PR resistance.

Second-line failures - genotype data

Of fifteen children with second-line virologic failure, 13 (87%) had serum available for genotype. Seven children were taking NNRTIs (54%), five (38%) lopinavir/r and one (8%) full-dose ritonavir, with background therapy predominantly AZT and ddI. Five children on NNRTI-based and two on LPV/r-based treatments had previously failed a full-dose ritonavir-based first-line regimen. Two children (15%) had been exposed to short-course AZT with single-dose NVP as pMTCT.

TAMs were more prevalent on second-line than first-line failure: in eight of 13 children (62%). M184V was present in three children (23%), but no K65R mutation was observed. NNRTI resistance mutations were present in nine (69%); the most prevalent mutations remained K103N and V106M. Five (38%) children had significant PR mutations (M46I, I54L, L/V82A/F, L90M), all children who had failed full-dose ritonavir-based therapy in first-line. ART regimen and corresponding mutations are displayed in Table 1b.

Second-line failures - predicted phenotypic susceptibility

Three (21%) of the 13 children failing second-line ART had no predicted resistance to any drug (see Table B, Supplemental Digital Content 1, <http://links.lww.com/INF/B441>). Compared to first-line failure there was a reduction in NRTI susceptibility (increased number of TAMs), but similar rates of NNRTI resistance. Five children with resistance to LPV/r had failed a ritonavir-based regimen in first-line ART (two on LPV/r-based and three on EFV-based second-line ART).

DISCUSSION

While the majority of the world's HIV-infected children live in sub-Saharan Africa there are few reports describing HIV-1 clade C resistance or outcomes of both first- and second-line failure in HIV-infected children in this region. This study corroborates findings of children failing first-line NNRTI-based [7,8,10] and PI-based ART [4,9,10].

The proportion of wild-type virus at first-line ART failure (19%) was consistent with other cohorts reporting between 9% [8] and 22% [7], which likely reflects inadequate ART adherence. TAMs and K65R were uncommon. M184V and NNRTI mutations (K103N and V106M) were found in over 50% failing first-line ART, probably due to the low genetic barrier to resistance of NNRTIs and 3TC compared to thymidine analogues.

Children failing second-line ART had mixed mutation patterns, varying from wild type virus (23%) to complex resistance to all currently available drug groups (38%). There were also more TAMs at second-line (62%) compared to first-line (19%) failure, likely due to a longer time on any failing therapy (first- or second-line). Explanations for failure on second-line based on these resistance patterns include persistent poor adherence or adherence to sub-optimal treatment regimens or dosing.

Multiple PR resistance mutations were seen in seven of 14 children who had used full-dose ritonavir at some point. Children receiving LPV/r had no PI resistance, consistent with data from previous reports [4,9,10]. Full-dose ritonavir was used for three years (2004 to 2007) in South Africa and is no longer used in most settings. Extensive PI resistance may thus be limited to the cohort of children treated in those years.

While the sample reflects diverse ART exposure, some important patterns are identifiable through genotyping. Full dose ritonavir should be avoided because of high rates of PI resistance. Currently recommended treatment regimens (i.e. NNRTI-based ART followed by LPV/r-based ART) have the most favorable resistance profile at second-line failure. Use of

genotype resistance testing for children failing second-line ART is an essential tool to allow for rational and efficient use of limited ART resources.

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Table 1

a: regimens and corresponding mutations for children failing first-line (n=37)					
First-line ART	Number of children: n	Median age at program entry: years (IQR)	Mean time to failure: years (IQR)	Prevalent mutations at first-line failure	
D4T or AZT / 3TC / NNRTI	24	7.45 (4.45–9.8)	2.4 (1.3–3.6)	M184V: 17 TAMs: D67N - 4; K70R - 2; T215F - 1. NNRTI: K101E/P - 4; K103N - 12; V106M - 11; V108V/I - 1; Y181Y/C - 1; G190A - 3. PR: T74S - 8.	
d4T or AZT / 3TC / LPV/r	9	1.2 (0.8–1.6)	2.8 (1.9–3.1)	M184V: 3 TAMs: L210W - 1. NNRTI: K103N - 3* PR: T74S - 1.	
d4T or AZT / 3TC / RTV	3	2.2 (0.6–2.6)	2.0 (1.8–3.5)	M184V: 2 TAMs: 0 NNRTI: 0; PR: D60E - 1; G73S - 1; V82A - 1;	
ddI / d4T / RTV	1	10.9 (10.9–10.9)	0.7 (0.7–0.7)	M184V: 0 TAMs: D67N - 1; K70R - 1; K219E - 1. NNRTI: 0. PR: L101V/F - 1; L241 - 1; E35D - 1; M361 - 1; M461 - 1; I54L - 1.	

b: regimens and corresponding mutations for children failing second-line (n=13).					
First-line ART	Second-line ART	Number of children: n	Median age at program entry: years (IQR)	Time to failure of second-line: years (IQR)	Prevalent mutations at second-line failure
d4T or AZT / 3TC / RTV	AZT / ddI / EFV	5	4.0 (3.0–9.0)	0.61 (0.55–1.98)	M184V: 1 TAMs: M41L - 3; D67N - 2; K70R - 1; L210W - 1; T215F - 4; K219E - 1 NNRTI: K101E/P - 1; K103N - 4; V106M - 4; Y181Y/C - 1; G190A - 1 Major PR mutations: L101V/F - 2; M361 - 2; I54L - 2; A71V/A/T - 2; T74S - 1; V82A - 2; L90M - 2.
d4T / 3TC / LPV/r		2	2.7 (2.0–3.4)	1.4 (1.16–1.63)	M184V: 0 TAMs: D67N - 2; K70R - 1; T215F - 1; K219E - 1 NNRTI: K101E/P - 2; K103N - 1; V106M - 2 PR: no major mutations
d4T / 3TC / EFV	AZT / ddI / LPV/r	3	9.0 (5.0–11.0)	0.84 (0.31–1.19)	M184V: 1 TAMs: 0 NNRTI: K103N - 2; P225H - 2. PR: no major mutations
d4T / ddI / RTV		1	10.9 (10.9–10.9)	2.18 (2.18–2.18)	M184V: 0 TAMs: D67N - 1; K70R - 1; K219E - 1 NNRTI: 0

b: regimens and corresponding mutations for children failing second-line (n=13).

First-line ART	Second-line ART	Number of children: n	Median age at program entry: years (IQR)	Time to failure of second-line: years (IQR)	Prevalent mutations at second-line failure
d4t / 3TC / RTV	d4t / 3TC / LPV/r	1	1.0 (1.0–1.0)	0.49 (0.49–0.49)	PR: L101V/F - 1; L241 - 1; E35D - 1; L33F/V - 1; M36I - 1; M46I - 1; I54L - 1;
AZT / 3TC / NVP	AZT / 3TC / RTV	1	1.0 (1.0–1.0)	0.65 (0.65–0.65)	M184V: 0 TAMs: 0 NNRTI: 0 PR: no major mutations
					M184V: 1 TAMs: D67N - 1 NNRTI: K103N: 1 PR: 32I - 1; M46I - 1; I54L - 1; L76V - 1; V82A - 1.

d4T: stavudine; AZT: zidovudine; 3TC: lamivudine; ddI: didanosine; LPV/r: lopinavir/ritonavir; RTV: ritonavir; TAM: thymidine analogue mutation; NNRTI: non-nucleoside reverse transcriptase inhibitor; PR: protease

* One of these children was exposed to pMTCT (AZT and NVP), the other two had unknown pMTCT status.