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Minimal Cross-resistance to Tenofovir in Children and Adolescents Failing ART Makes Them Eligible for Tenofovir-Lamivudine-Dolutegravir Treatment

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Background: Fixed-dose combination of dolutegravir (DTG) with tenofovir disoproxil fumarate (TDF) and lamivudine (3TC) likely improves adherence and has a favorable resistance profile. We evaluated predicted efficacy of TLD (TDF-3TC-DTG) in children and adolescents failing abacavir (ABC), zidovudine (AZT), or TDF containing regimens.

Methods: Drug resistance mutations were analyzed in a retrospective dataset of individuals <19 years of age, failing ABC (n = 293) AZT (n = 288) or TDF (n = 69) based treatment. *Pol* sequences were submitted to Stanford HIVdb v8.9. Genotypic susceptibility scores were calculated for various DTG-containing regimens.

Results: Genotypes were assessed for 650 individuals with a median age of 14 years (IQR 10-17 years). More individuals failed a protease inhibitor (PI)-based (78.3%) than a non-nucleoside reverse transcriptase inhibitors (NNRTI)-based (21.7%) regimen. Most individuals in the AZT group (n = 288; 94.4%) failed a PI-based regimen, compared with 71.0% and 64.2% in the TDF (n = 69) and ABC group (n = 293). Genotypic sensitivity scores <2 to TLD were observed in 8.5% and 9.4% of ABC- and AZT-exposed individuals, compared with 23.2% in the TDF group. The M184V mutation was most often detected in the ABC group (70.6%) versus 60.0% and 52.4% in TDF and AZT groups. The presence of K65R was rare (n = 13, 2.0%) and reduced TLD susceptibility was commonly caused by accumulation of nucleoside reverse transcriptase inhibitor (NRTI) mutations.

Conclusions: Cross-resistance to TDF was limited, further reducing concerns about use of transition to TLD in children and adolescents. The NADIA trial has subsequently shown that patients failing a TDF/3TC/EFV regimen can safely be transitioned to a TLD regimen but we do not have data for patients failing an ABC/3TC/NNRTI or PI regimens. Frequent virological monitoring is recommended after switch to DTG, especially in children continuing ABC in the backbone. Clinical studies correlating predicted resistance with clinical outcomes, especially in settings without access to genotyping, are required.

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INTRODUCTION

South Africa has made great strides towards achieving the UNAIDS 90-90-90 targets, including a 40% reduction in new HIV infections in 2019 compared with 2010.[1] Despite this great progress, the country still accounts for 7.8 million people living with HIV, including 310,000 children under the age of 15.[2] In addition, there is a substantial gap between ART coverage in adolescent and adult women and men (78% and 63%, respectively) and in children <15 years (47%). Likewise, viral suppression rates in those on ART range from 66% across all ages to only 33% in children.[2] Dolutegravir (DTG) combined with lamivudine (3TC) and tenofovir disoproxil fumarate (TDF) is a single tablet regimen (TLD), which is being rolled-out in South Africa for adults and adolescents. Adolescents older than 10 years and weighing at least 35 kg qualify for switching to TLD. Despite the high genetic barrier of DTG,[3] clinical trials with DTG monotherapy have also shown that the development of DTG resistance is a tangible risk when treatment failure occurs.[4] Four patients (1%) in the SAILING study who were treated with DTG and other drugs without full activity also developed DTG resistance upon failure.[5] A recent sentinel surveillance study among children and adolescents experiencing ART failure in South Africa showed that 70.5% of children and adolescents presented with resistance to the nucleoside reverse transcriptase inhibitor (NRTI) class.[6]

Abacavir (ABC) is commonly used in pediatric and adolescent ART and can possibly select for drug resistance mutations conferring cross-resistance to TDF. Historically, stavudine (d4T) and zidovudine (AZT) have been frequently prescribed in children and adolescents. Both of these NRTIs frequently select for thymidine analogue mutations (TAMs), the accumulation thereof could also reduce susceptibility to TDF. Subsequent to our study being done, the NADIA trial showed that TLD after TDF-FTC-EFV (efavirenz) failure was superior to AZT-3TC-DTG although that was not known at the time of our study.[7] The clinical impact of switching treatment experienced patients to DTG-based regimens was poorly understood at the time, therefore knowing the frequency of reduced predictive efficacy to TLD in children and adolescents is still useful in low- and middle-income countries (LMICs) where genotypic testing before treatment switch is often not available.

In this study, we assessed the genotypic susceptibility score (GSS) for TLD in children and adolescents failing ABC-, AZT-, and TDF-based regimens.

MATERIALS AND METHODS

Study Population

We conducted a retrospective analysis of all HIV drug resistance data obtained from HIV-infected patients ≤19 years of age

failing ABC-, AZT-, or TDF-based regimens who had an HIV drug resistance test performed at Charlotte Maxeke Johannesburg Academic Hospital Genotyping Laboratory between January 2017 and December 2018. Drug resistance testing was available as part of routine care, only if requested by the treating clinician for patients with confirmed virological failure. At the time of study, guidelines recommended resistance testing for patients failing PI-based regimens only; however, resistance testing for NNRTI-based failures could be requested at clinician's discretion. Demographic and clinical data such as age, gender, viral load, and antiretroviral treatment regimen were collected from laboratory request forms. Sequences were excluded if the treatment regimen at time of genotyping was unknown, if the patient was treated with only 1 or 2 drugs; or if the NRTI backbone contained a combination of ABC, AZT, and TDF.

Pol sequences were generated using validated population-based in-house genotyping methods, depending on the time of sampling.[8] Sequences were aligned using cd-hit to detect cross-contamination and omit duplicates from analyses.[9] For every cluster with a similarity of 97.5% or higher, which indicates sequences originating from the same individual, the cumulative resistance profile for that patient was used for the analysis.

The Stanford HIVdb v8.9 tool (https://hivdb.stanford.edu/hivdb/by-sequences/) was used to identify drug resistance mutations (DRMs) and interpret resistance profiles, categorized as susceptible (susceptible and potential low-level resistance), intermediate (low-level resistance and intermediate resistance), or high-level resistance. GSS were calculated by assigning a score of 0 to high-level resistance; 0.5 to intermediate resistance and 1 for susceptible predictions. A GSS < 2 therefore indicates a regimen with less than 2 active drugs. Subtyping was performed using the Rega HIV subtyping tool v3.0 (http://dbpartners.stanford.edu:8080/RegaSubtyping/stanford-hiv/typingtool/).

The *pol* nucleotide sequences were submitted to GenBank using Bankit (https://www.ncbi.nlm.nih.gov/WebSub/); accession numbers: OK091675-OK091684, OK091686-OK091696, OK091698-OK091704, OK091706-OK091721, OK091723-OK091729, OK091731-OK091735, OK091737-OK091797, OK091799-OK091804, OK091806-OK091822, OK091824-OK091841, OK091843-OK091848, OK091850-OK091854, OK091856-OK091858, OK091860-OK091877, OK091879-OK091898, OK091900-OK091901, OK091905-OK091924, OK091926-OK091946, OK091948-OK091978, OK091980-OK092109, OK092111-OK092131, OK092133-OK092144, OK092146-OK092177, OK092179- OK092212, OK092214-OK092291 and ON088503-ON088596.

Statistical Analysis

First, we summarized demographic and clinical characteristics, drug resistance profiles and GSS of children and adolescents, stratified by failure on an ABC-, AZT- or TDF-based regimen as well as by PI-based versus NNRTI-based treatment, using proportions for categorical variables and median and interquartile range (IQR) for continuous variables.

Second, to compare drug resistance profiles and genotypic sensitivity scores between failure on an ABC-, AZT- or TDF-based regimen, we used the Mann-Whitney *U* test for nonparametric data, Student's *t* test for parametric or normally distributed data, and the χ^2 test (or Fisher's exact test for sparse data) for proportions. A *P* value <0.05 was considered statistically significant.

Third, we graphically present the resistant profiles of children and adolescents, experiencing treatment failure by regimen type.

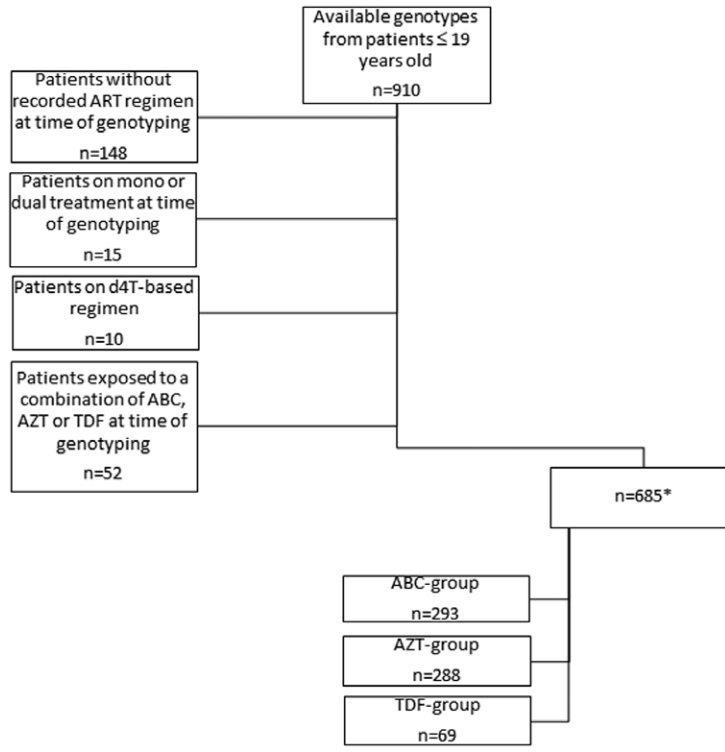
Fourth, we determined if prior ART exposure was associated with drug resistant mutations (eg, any TAM, 3 or more TAMs, or intermediate/high-level resistance to TDF) and genotypic sensitivity score <2 to TLD. To do this, we used a log-binomial regression model to estimate the crude Relative Risk (RR) with the corresponding 95% confidence interval (CI). Prior exposure to antiretroviral treatment was collected from laboratory request forms and grouped as exposure to ABC, d4T, didanosine (ddI), TDF or AZT alone or in combination (ie, any combination of 2 or more drugs; d4T, TDF, ddI, AZT or ABC). Variables with a *P* value less than 0.25 in the univariate analysis along with a priori variables (eg, age, gender, last HIV viral load, antiretroviral treatment regimen) were included in the final multivariate model.

Ethics Statement

This study was conducted in accordance with the Declaration of Helsinki and national and institutional standards. Ethical clearance was obtained by the Research on Human Subjects (Medical) Committee at the University of the Witwatersrand (Clearance Number M181158). The study was a retrospective review of programmatic data and a waiver of informed consent was granted to retrospectively review these records.

RESULTS

Between January 2017 and December 2018, a total of 910 specimens were genotyped. Specimens received from patients without recorded ART exposure at time of genotyping (n = 148), or exposed to mono or dual therapy (n = 15) at time of genotyping were excluded. Fifty-two patients were excluded because they were exposed to a combination of ABC, AZT or TDF at time of genotyping. Finally, patients failing stavudine containing regimens (n = 10) were excluded caused by small sample size (Fig. 1). Phylogenetic analysis was performed on all remaining sequences (n = 685). For patients with more than 1 genotype, the cumulative resistance profile obtained from multiple sequences was used for analysis. The final cohort consisted of 650 unique patients (48.5% male), including 293, 288 and 69 patients experiencing failure to ABC, AZT and TDF-based regimens respectively, at time of resistance testing. The median age was 14 years (IQR 10–17 years), children and adolescents in the TDF group were older compared with those in the AZT group and the ABC group (Table 1). The median HIV viral load was 4.8 $\log_{10}$ copies/mL (IQR: 4.2–5.3). A higher proportion of patients experienced failure to a protease inhibitor (PI) based regimen in the AZT group (94.4%) compared with 71.0% and 64.2% in the TDF and ABC group ($P < 0.0001$). Most patients were infected with subtype C (99.2%); 4 patients were infected with subtype A and 1 patient with an A/D recombinant. Only 103 patients (15.8%) presented without any DRMs. The absence of DRMs was more commonly observed in children and adolescents experiencing failure in the AZT group (20.5%) compared with those experiencing failure in the ABC- or TDF group (11.3% and 15.9%, $P = 0.010$, Tables 2 and 3). Similarly, absence of DRMs was more often seen in patients experiencing failure in the PI-group (19.6%) compared with those experiencing failure in the NNRTI-group at time of genotyping (2.1%, $P < 0.0001$, Tables 2 and 3). Likewise, the detection of NRTI, NNRTI and dual class NRTI+NNRTI resistance was less likely in individuals experiencing failure in the AZT- or PI-group (Tables 2 and 3). The M184V mutation was frequently observed across all patients (n = 398, 61.2%), but less often detected in the AZT group (n = 151, 52.4%) compared with the ABC group (n = 207, 70.6%, $P < 0.0001$) and compared with the TDF group (n = 40, 60.0%, $P = 0.0453$). Patients failing PI-based regimens presented less often with M184V (n = 275, 54.0%) versus those failing NNRTI-based regimens at time of genotyping (n = 123, 87.2%, $P < 0.0001$).



*For 33 patients more than one HIVDR test was performed during the study period (31 patients with 2 sequences, 2 patients with 3 sequences). For these patients, the treatment status at the latest time point was considered and the cumulative resistance profile was included in analysis.

FIGURE 1. Flow chart documenting exclusion criteria and the number of individuals included in the study population. ABC indicates abacavir; ART, antiretroviral treatment; AZT, zidovudine; d4T, stavudine; TDF, tenofovir.

Accumulation of 3 or more NRTI mutations was more common in the ABC- (33.5%) and the TDF group (26.1%) versus the AZT group (15.3%, $P < 0.0001$) and more common in those failing NNRTI-based regimens (54.6%) versus those failing PI-based regimens (16.3%, $P < 0.0001$) (Tables 2 and 3). The presence of 3 or more TAMs did not differ between the ABC group (3.4%) and the

TABLE 1. Demographic and Clinical Characteristics of Children and Adolescents Experiencing Failure on Abacavir, Tenofovir or Zidovudine-based Antiretroviral Treatment

	All (n = 650)	ABC group (n = 293)	AZT group (n = 288)	TDF group (n = 69)
Sex male n (%)	315 (48.5)	147 (50.2)	137 (47.6)	31 (44.9)
Age median (IQR), y	14 (10–17)	11 (6–15)	15 (13–17)	18 (16–19)
<5, n (%)	65 (10.0)	52 (17.7)	13 (4.5)	0 (0)
5–9, n (%)	84 (12.9)	57 (19.5)	25 (8.7)	2 (2.9)
10–14, n (%)	186 (28.6)	100 (34.1)	82 (28.5)	4 (5.8)
15–19, n (%)	315 (48.5)	84 (28.7)	168 (58.3)	63 (91.3)
Log HIVVL median (IQR)	4.8 (4.2–5.3)	4.7 (4.2–5.3)	4.9 (4.2–5.4)	4.9 (4.1–5.4)
ART exposure at time of HIVDR testing				
PI-based, n (%)	509 (78.3)	188 (64.2)	272 (94.4)	49 (71.0)
NNRTI-based, n (%)	141 (21.7)	105 (35.8)	16 (5.6)	20 (29.0)
Prior NRTI exposure				
Unknown, n (%)	281 (43.2)	193 (65.9)	73 (25.3)	15 (21.7)
TDF, n (%)	34 (5.2)	7 (2.9)	20 (6.9)	–
ddI, n (%)	24 (3.7)	4 (1.4)	17 (5.9)	3 (4.3)
d4T, n (%)	162 (24.9)	52 (17.7)	79 (27.4)	31 (44.9)
ABC, n (%)	204 (31.4)	–	137 (47.6)	26 (37.7)
AZT, n (%)	79 (12.2)	18 (6.1)	–	20 (29.0)

Unknown NRTI exposure may include children and adolescents on first-line ART (no prior ART exposure) or undisclosed prior ART exposure in children/adolescents on second-line ART.

ABC indicates abacavir; ART, antiretroviral treatment; AZT, zidovudine; d4T, stavudine; ddI, didanosine; HIVDR, HIV drug resistance; HIVVL, HIV viral load (log_{10} RNA copies/mL); IQR, interquartile range; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; TDF, tenofovir.

TABLE 2. Drug resistance profiles and genotypic susceptibility scores in children and adolescents experiencing failure by NRTI exposure group

	All (n = 650)	ABC-group (n = 293)	AZT-group (n = 288)	TDF-group (n = 69)	*P*
Resistance class profiles					
No DRMs	103 (15.8%)	33 (11.3%)	59 (20.5%)	11 (15.9%)	**0.010**
NRTI resistance	422 (64.9%)	217 (74.1%)	162 (56.3%)	43 (62.3%)	**<0.001**
NNRTI resistance	467 (71.8%)	222 (75.8%)	193 (67.0%)	52 (75.4%)	**0.050**
NRTI + NNRTI resistance	349 (53.7%)	181 (61.8%)	129 (44.8%)	39 (56.5%)	**<0.001**
PI resistance	84 (12.9%)	41 (14.0%)	35 (12.2%)	8 (11.6%)	0.756
NRTI DRMs					
None	225 (34.6%)	75 (25.6%)	124 (43.1%)	26 (37.7%)	**<0.001**
1	199 (30.6%)	84 (28.7%)	97 (33.7%)	18 (26.1%)	0.292
2	66 (10.2%)	36 (12.3%)	23 (8.0%)	7 (10.1%)	0.229
≥3	160 (24.6%)	98 (33.5%)	44 (15.3%)	18 (26.1%)	**<0.001**
TAMs					
None	548 (84.3%)	258 (88.1%)	233 (80.9%)	57 (82.7%)	0.056
1–2	74 (11.4%)	25 (8.5%)	42 (14.6%)	7 (10.1%)	0.068
≥3	28 (4.3%)	10 (3.4%)	13 (4.5%)	5 (7.2%)	0.360
GSS<2					
TDF-3TC-DTG	63 (9.5%)	22 (7.4%)	25 (8.7%)	16 (23.2%)	**0.001**
ABC-3TC-DTG	399 (61.4%)	208 (71.0%)	151 (52.4%)	40 (58.0%)	**<0.001**
AZT-3TC-DTG	69 (10.6%)	20 (6.8%)	39 (13.5%)	10 (14.5%)	**0.003**

3TC indicates lamivudine; ABC, abacavir; AZT, zidovudine; DRM, drug resistance mutation; DTG, dolutegravir; GSS, genotypic susceptibility score; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor TAM, thymidine analogue mutation; TDF, tenofovir.

Statistically significant differences are indicated in bold.

TABLE 3. Drug Resistance Profiles and Genotypic Susceptibility Scores by or Anchor Drug Class

	All (n = 650)	PI-group (n = 509)	NNRTI-group (n = 141)	*P*
Resistance class profiles				
No DRMs	103 (15.8%)	100 (19.6%)	3 (2.1%)	**<0.0001**
NRTI resistance	422 (64.9%)	295 (58.0%)	127 (90.1%)	**<0.0001**
NNRTI resistance	467 (71.8%)	330 (64.8%)	137 (97.2%)	**<0.0001**
NRTI+ NNRTI resistance	349 (53.7%)	223 (43.8%)	126 (89.4%)	**<0.0001**
PI resistance	84 (12.9%)	78 (15.3%)	6 (4.3%)	**0.001**
NRTI DRMs				
None	225 (34.6%)	210 (41.3%)	15 (10.6%)	**<0.0001**
1	199 (30.6%)	179 (35.2%)	20 (14.2%)	**<0.0001**
2	66 (10.2%)	37 (7.3%)	29 (20.6%)	**<0.0001**
≥3	160 (24.6%)	83 (16.3%)	77 (54.6%)	**<0.0001**
TAMs				
None	548 (84.3%)	432 (84.9%)	116 (82.3%)	0.478
1–2 TAMs	74 (11.4%)	54 (10.6%)	20 (14.2%)	0.252
≥3 TAMs	28 (4.3%)	23 (4.5%)	5 (3.5%)	0.602
GSS<2				
TDF-3TC-DTG	63 (9.7%)	37 (7.3%)	26 (18.4%)	**0.001**
ABC-3TC-DTG	399 (61.4%)	275 (54.0%)	124 (87.9%)	**<0.0001**
AZT-3TC-DTG	69 (10.6%)	52 (10.2%)	17 (12.1%)	0.687

3TC indicates lamivudine; ABC, abacavir; AZT, zidovudine; DRM, drug resistance mutation; DTG, dolutegravir; GSS, genotypic susceptibility score; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor TAM, thymidine analogue mutation; TDF, tenofovir.

Statistically significant differences are indicated in bold.

AZT group (4.5%) or the TDF group (7.2%); neither was a difference observed between patients failing PI-based regimens (4.5%) and NNRTI-based regimens (3.5%) (Tables 2 and 3).

From bivariate or multivariate logistic regression, prior exposure to either AZT, TDF or ddI (aRR 2.00, 95% CI: 1.00-4.01), male gender (aRR 1.45, 95% CI: 1.01-2.09) and AZT exposure at time of genotyping (aRR 2.06, 95% CI: 1.29-3.20) were associated with the presence of at least 1 TAM (Table 4). Failing a PI-based regimen at time of genotyping was associated with a lower prevalence of TAMs (aRR 0.56, 95% CI: 0.34-0.90). The presence of at least 3 TAMs was not associated to any of the variables tested (Table 4). Patients failing TDF regimens at time of genotyping had a 2.61-fold higher chance to present with intermediate or high-level resistance to TDF (aRR 2.61, 95% CI: 1.12-6.11) and a 2.98-fold risk to have a TLD GSS<2 (aRR 2.98, 95% CI: 1.20-7.41). HIV viral load above 100 000 copies/mL at time of genotyping also increased the risk for TDF resistance (aRR 3.18, 95% CI: 1.41-7.12) and to present with a TLD GSS<2 (aRR 2.87, 95% CI: 1.26-6.53). PI exposure at time of genotyping has a protective effect to the development of TDF resistance (aRR 0.35, 95% CI: 0.20-0.64) and a TLD GSS<2 (aRR 0.32, 95% CI: 0.18-0.59).

Predicted NRTI resistance profiles are depicted in Figure 2. Genotypic sensitivity scores <2 to TLD were only detected in 63 patients (9.7%); there was no statistical difference between patients

TABLE 4. Factors Associated With Drug Resistant Mutations and Genotypic Sensitivity Score <2 to TLD in Children and Adolescents Failing on an Abacavir or Zidovudine-based Treatment (n = 617)

	Proportion	Crude RR 95% CI	Adjusted RR 95% CI	Proportion	Crude RR 95% CI	Adjusted RR 95% CI
	Any TAM (n = 102)			**3 or more TAMS (n = 28)***		
Prior exposure						
No/unknown prior	38/286 (13.3%)	1.0	1.0	12/286 (4.2%)	1.00	1.0
ABC alone	18/132 (13.6%)	1.03 (0.61–1.73)	0.90 (0.52–1.56)	3/132 (2.3%)	0.54 (0.16–1.87)	0.56 (0.16–2.00)
D4T alone	15/87 (17.2%)	1.30 (0.75–2.24)	1.45 (0.82–2.56)	5/87 (5.8%)	1.37 (0.50–3.78)	1.31 (0.47–3.65)
Combination (≥2)†	23/111 (20.7%)	1.77 (0.90–3.48)	1.49 (0.90–2.48)	7/111 (6.3%)	1.50 (0.61–3.72)	1.57 (0.64–3.90)
ddI/TDF/AZT alone	8/34 (23.5%)	1.56 (0.98– 2.49)	**2.00 (1.00–4.01)**	1/34 (2.9%)	0.70 (0.09–5.22)	0.83 (0.11–6.28)
Gender						
Female	44/335 (13.1%)	1.0	1.0	10/335 (3.0%)	1.0	1.0
Male	58/315 (18.4%)	1.40 (0.98–2.01)	**1.45 (1.01–2.09)**	18/315 (5.7%)	1.91 (0.90–4.08)	1.87 (0.87–3.99)
Current regimen						
ABC	35/293 (12.0%)	1.0	1.0	10/293 (3.4%)	1.0	
AZT	55/288 (19.1%)	**1.60 (1.08–2.36)**	**2.06 (1.29–3.20)**	13/288 (7.3%)	1.32 (0.59–3.00)	
TDF	12/69 (17.4%)	1.46 (0.80–2.66)	1.13 (0.59–2.27)	5/69 (7.3%)	2.12 (0.75–6.01)	
PI exposure at time of genotyping						
No	25/141 (17.7%)	1.0	1.0	5/141 (3.6%)	1.0	
Yes	77/509 (15.1%)	0.85 (0.57–1.29)	**0.56 (0.34–0.90)**	23/509 (4.5%)	1.27 (0.49–3.29)	
Age (y)						
<10	21/147 (14.3%)	1.0	1.0	4/147 (2.7%)	1.0	1.0
10–14	31/181 (17.1%)	1.11 (0.71–1.73)	0.95 (0.58–1.57)	10/181 (5.5%)	1.35 (0.56–3.25)	1.37 (0.57–3.32)
15–19	37/249 (14.9%)	0.96 (0.62–1.48)	0.75 (0.45–1.25)	9/249 (3.6%)	0.88 (0.35–2.19)	0.96 (0.38–2.41)
Last HIV viral load						
<10,000	17/126 (13.5%)	1.0	1.0	3/126 (2.4%)	1.0	
10,000–100,000	35/254 (13.8%)	1.04 (0.61–1.78)	1.10 (0.64–1.87)	10/254 (3.9%)	1.67 (0.47–5.95)	
>100,000	50/269 (18.6%)	1.40 (0.84–2.34)	1.53 (0.92–2.55)	15/269 (5.6%)	2.36 (0.70–8.00)	
Prior exposure	**Intermediate or high-level resistance to TDF (n = 69)‡**			**TLD GSS <2 (n = 63)‡**		
No/unknown prior	29/286 (10.1%)	1.0	1.0	26/284 (4.0%)	1.0	1.0
ABC alone	9/132 (6.8%)	0.67 (0.33–1.38)	0.79 (0.35–1.77)	8/131 (6.1%)	0.67 (0.31–1.43)	0.78 (0.33–1.84)
D4T alone	9/87 (10.3%)	1.02 (0.50–2.07)	1.19 (0.54–2.62)	8/86 (9.3%)	1.02 (0.48–2.16)	1.19 (0.52–2.72)
ddI/TDF/AZT alone	18/111 (16.2%)	1.60 (0.93–2.76)	1.61 (0.83–3.13)	17/110 (15.5%)	1.69 (0.95–2.99)	1.68 (0.84–3.34)
Combination (≥2)†	4/34 (11.8%)	1.16 (0.43–3.10)	1.49 (0.49–4.50)	4/34 (11.8%)	1.29 (0.48–3.46)	1.68 (0.55–5.14)
Gender						
Female	28/335 (8.4%)	1.0	1.0	25/333 (7.5%)	1.0	1.0
Male	41/315 (13.0%)	**1.56 (1.00–2.46)**	1.48 (0.91–2.40)	38/312 (12.2%)	**1.62 (1.00–2.62)**	1.53 (0.92–2.55)
Current regimen						
ABC	25/293 (8.5%)	1.0	1.0	22/290 (7.8%)	1.0	1.0
AZT	27/288 (9.4%)	1.10 (0.65–1.85)	1.59 (0.84–3.03)	25/286 (8.7%)	1.15 (0.67–2.00)	1.74 (0.88–3.43)
TDF	17/69 (24.6%)	**2.89 (1.65–5.04)**	**2.61 (1.12–6.11)**	16/69 (23.2%)	**3.06 (1.70–5.50)**	**2.98 (1.20–7.41)**
PI exposure at time of genotyping						
No	27/141 (19.2%)	1.0	1.0	26/141 (18.4%)	1.0	1.0
Yes	42/509 (8.3%)	**0.43 (0.28–0.67)**	**0.35 (0.20–0.64)**	37/504 (7.3%)	**0.40 (0.25–0.63)**	**0.32 (0.18–0.59)**
Age (y)						
<10	11/147 (7.5%)	1.0	1.0	9/145 (6.2%)	1.0	1.0
10–14	23/181 (12.7%)	1.0 (0.60–1.67)	1.30 (0.62–2.74)	22/180 (12.2%)	1.07 (0.62–1.82)	1.48 (0.66–3.29)
15–19	18/249 (7.2%)	**0.57 (0.32–0.99)**	0.88 (0.40–1.94)	16/247 (6.5%)	0.57 (0.31–1.03)	0.95 (0.40–2.22)
Last HIV viral load						
<10,000	7/126 (5.6%)	1.0	1.0	7/126 (5.6%)	1.0	1.0
10,000–100,000	22/254 (8.7%)	1.57 (0.69–3.58)	1.66 (0.71–3.90)	21/253 (8.3%)	1.51 (0.66–3.45)	1.60 (0.67–3.77)
>100,000	40/269 (14.9%)	**2.70 (1.24–5.85)**	**3.18 (1.41–7.12)**	35/265 (13.2%)	**2.40 (1.10–5.24)**	**2.87 (1.26–6.53)**

*one predictor variable for every ten outcomes, to keep the risk of overfitting low.

†Any combination of 2 or more drugs (d4T, TDF, ddI, AZT, ABC); bold font represents a $P < 0.05$.

‡PROC GENMOD used to perform Poisson regression.

3TC indicates lamivudine; ABC, abacavir; AZT, zidovudine; CI, confidence interval; DTG, dolutegravir; GSS, genotypic susceptibility score; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor TAM, thymidine analogue mutation; RR, relative risk; TDF, tenofovir.

failing ABC (7.5%) or AZT-based regimens (8.7%), but a quarter of individuals failing TDF regimens presented with a TLD GSS<2 (Tables 2 and 3). Patients failing NNRTI-based regimens were more likely to have a GSS<2 (18.4%) compared with those failing PI-based regimens (7.3% $P = 0.001$). Combining ABC-3TC with DTG did not yield promising GSS outcomes: more than half of the patients experiencing failure to AZT and TDF-based regimens (52.4% and 58.0%) were predicted to have a GGS<2 and 71.0% of the children and adolescents experiencing ABC-based ART failure were expected to retain limited activity from ABC-3TC-DTG. However, based on genotypic predictions, a combination of AZT-3TC-DTG could possibly offer a suitable alternative to TLD in 89.4% of the children and adolescents (Tables 2 and 3).

Eleven patients presented with high-level resistance to TDF (1.7%), in 6 cases caused by K65R, in the other 5 cases caused by 4 to 6 TAMs. Partial activity to TDF was lost in 58 patients (8.9%), most often caused by the presence of 2-4 TAMs (n = 38). Other patients with reduced TDF susceptibility presented with K70ENT (n = 5), Y115F (n = 4), K70E+Y115F (n = 2) K65R+M184V (n = 7) K65N (n = 1) or T69 insertion (n = 1).

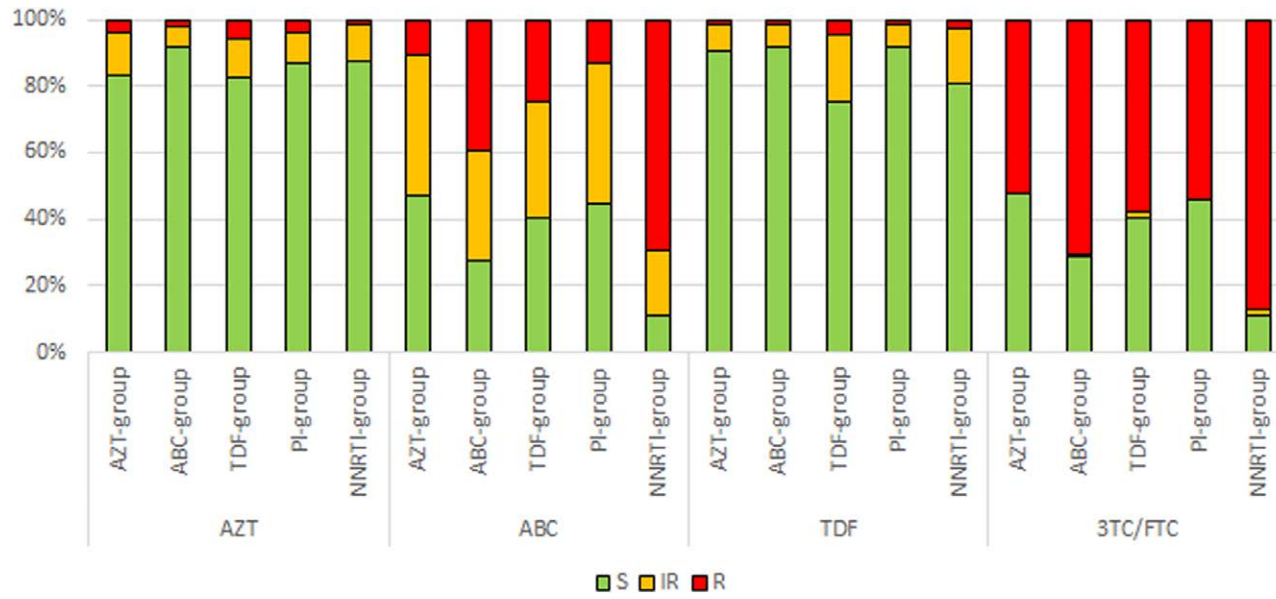


FIGURE 2. Predicted NRTI resistance profiles in children and adolescents experiencing treatment failure. 3TC, lamivudine; ABC, abacavir; AZT, zidovudine; FTC, emtricitabine; IR, intermediate resistance; NNRTI, non-nucleoside reverse transcriptase inhibitors; PI, protease inhibitors; R, resistance; S, susceptible; TDF, tenofovir.

DISCUSSION

In 2019, most of the WHO priority countries had adopted DTG as first-line therapy in children weighing more than 20 kg.[10] However, there is still some concern that switching patients experiencing ART failure to TLD carries a potential risk of the development of dolutegravir resistance, especially in resource-limited settings where virological monitoring and genotypic resistance testing is limited.[11] The results of our study indicate that resistance is common in children and adolescents experiencing treatment failure who had a genotypic test, with 84.2% of them presenting with at least 1 DRM, which is consistent with recent findings from a sentinel survey in South Africa.[6] The use of PIs was more common in the AZT group compared with the ABC- and TDF-groups, which is in line with the treatment guidelines during the study period that recommended ABC-3TC with either EFV or LPV/r, depending on age as a first-line regimen, but AZT-3TC-LPV/r as the standard second-line regimen. TDF regimens were generally recommended in older children in combination with EFV or LPV/r depending on previous ART exposure.

Overall, less resistance was observed in the AZT group versus the ABC and TDF groups and in the PI-group versus the NNRTI-group. While the mechanism for this is poorly understood, we speculate that it may be related to poorer adherence in AZT and PI-exposed patients. Because of the low genetic barrier to develop M184V/I, the presence of this mutation is expected in children failing treatment but with ongoing drug pressure. The absence of M184V/I would suggest poorer adherence. Other mechanisms such as particular resistance pathways related to AZT pressure may also account for absence of M184V/I. There are several explanations for poorer adherence in the AZT group. First, AZT is administered twice daily, which increases the risk for poor adherence. Second, most children and adolescents received AZT in combination with LPV/r, which are known for gastrointestinal side effects, might impact on treatment adherence. Third, about 60% of patients in the AZT group were 14 years or older and adolescents seem to struggle more often to maintain good treatment adherence.[12]

The K65R mutation is known as a signature mutation under TDF pressure[13]; however, this mutation can also be selected under ABC drug pressure.[14, 15] And so, there is concern for cross-resistance when patients, who experience failure to an ABC-based regimen, are switched to TLD. In addition, subtype C viruses, which is the predominant subtype in South Africa, are more likely to develop K65R.[15-17] Nevertheless, K65R was only seen in 4 ABC-exposed patients (1.4%), including 3 patients presenting with K65R+M184V yielding intermediate resistance to TDF. In the AZT group, K65K/R was observed in 2 adolescents, one of whom also presented with M184M/V and had known prior exposure to TDF. The other patient presented with K65K/R+M184M/V+A62A/V+D67D/N but prior ART exposure was unknown. Overall, only 11 patients (1.7%) presented with high-level resistance to TDF and an additional 8.9% (n = 58) presented with partial loss of activity to TDF. The limited cross-resistance to TDF resulted in less than 10% of children and adolescents with a TLD GSS<2. No significant difference in reduced GSS was observed between the ABC and AZT groups; however, a TLD GSS<2 was seen in a quarter of patients failing TDF-based regimens. The frequency of patients with TLD GSS<2 was also less common in patients failing PI regimens at time of genotyping. This is in line with findings from the PENPACT study, which showed that accumulation of NRTI resistance was less common in children being treated with PI-based regimens.[18] In most cases, the reduced GSS score was attributed to the accumulation of NRTI mutations. The accumulation of NRTI mutations was least common in the AZT group, which can potentially be linked to the protective effect of PIs or poorer adherence as discussed earlier. The presence of at least 1 TAM in 11.9% of children and adolescents failing ABC containing regimens indicates prior exposure to thymidine analogues in this group. Although 25.3% of these children had recorded prior exposure to thymidine analogues, for 65.9% of these children no prior history was recorded. The lack of recorded prior ART exposure might indicate that these patients were on a first-line regimen at the time of genotyping, or that the treatment history was incomplete on the laboratory request form.

Although the TLD GSS is favorable in most children and adolescents in our study, the accumulation of NRTI mutations is an important risk factor. The risk of developing resistance increases with incomplete treatment adherence.[19] Moreover, limited availability of

pediatric formulations, unexpected drug switches at times of inte rupted drug supply, and poor tolerance of some drugs are other contributors to insufficient drug pressure, which may in turn lead to accumulation of resistance.[20, 21] It has also been shown that the lack of frequent viral load monitoring and appropriate clinical action when virological failure is detected, increase the risk of accumulation of drug resistance mutations.[22, 23] Single tablet regimens have been shown to improve treatment adherence and thereby reduce the risk for developing resistance[24]; which is 1 of the reasons why TLD is such an attractive treatment option, in addition to the high genetic barrier to resistance, the potency and limited side effects. In those children and adolescents where contraindications prevent the use of TLD, our study has shown that AZT-3TC-DTG would be an alternative regimen. In adults, the NADIA trial has shown that AZT-based regimens are inferior to TLD in terms of viral suppression with an increased risk of DTG resistance[5,25]; it is however unknown if the same applies in children and adolescents with pre-existing NRTI resistance.

Although GSS predictions can provide an indication of what to expect when patients are switched to a certain regimen, clinical outcome data are required to assess the impact of a partially active backbone in combination with DTG. In adults, the EVER-EST, SELECT and SECOND-LINE trials have shown that with a potent drug like LPV/r, the backbone regimen does not matter as much as initially thought.[26-30] The same might be true for DTG-containing regimens given the incredible efficacy as indicated by the NADIA trail.[5,25] Although these results are reassuring for use of TLD in treatment experienced patients, the acquisition of DTG resistance needs to be monitored. Furthermore, there are important differences when comparing NADIA with our population: the study only had 3 participants under the age of 18; the K65R mutation was very prevalent (50%) suggesting extensive prior TDF exposure; and there was limited exposure to ABC, ddI and PIs. In the ART-IST trial, switching adults who failed a TDF and NNRTI-based first-line regimen to TLD yielded good virological suppression rates (85%) at 24 weeks, without development of DTG resistance in those failing TLD.[31] However, no children were included in this trial, the follow-up was short (24 weeks) and the baseline viral load at switch was low (10 580 copies/mL). [31] The Odyssey trial showed infrequent virological failure in children who were switched to a DTG-containing second-line regimen; however, a handful of patients developed DTG resistance upon failure. Three of the 4 participants with DTG mutations were receiving AZT and lamivudine twice daily.[32] While GSS likely contributes to the risk of virological failure, both in adults and children, other factors such as adherence might have an important impact on the effectiveness of DTG-based second-line regimens. Therefore, more clinical trials and real-life data are required to confirm the relevance of previously acquired NRTI mutations, in the context of adherence, when switching to TLD and more frequent viral load monitoring might be required.

The presented study has some limitations. We present observational data, which are prone to a wide variety of potential biases, including referral bias. However, the age distribution is similar to that observed in the latest South African sentinel surveillance study.[6] Likewise, the younger population in the ABC group reflect the treatment recommendations in South Africa. Children and adolescents experiencing failure to NNRTI-based regimens are underrepresented in this study as guidelines indicate that genotyping is only recommended in patients experiencing failure to PI-based regimens. Some children failing NNRTI-based treatment had a genotype done based on the clinician's discretion. The retrospective nature of the data did not allow us to assess the impact of treatment duration. However, at the time of study, genotyping was recommended upon confirmed virological failure, when patients had been treated with a second-line regimen for at least 1 year and relevant adherence issues were addressed. In reality, it is likely that most patients had been exposed to PI-based regimens for at least 2 years, before genotyping. Prior treatment exposure information was not always available and therefore we were unable to classify children as first or second-line failures. Finally, resistance mutations were detected by population-based Sanger sequencing, the sensitivity of this method may have underestimated the prevalence of minority drug resistance mutations, particularly in patients who were noncompliant, or have been exposed to multiple antiretroviral drugs over time. It is therefore possible that archived mutations were missed in this analysis.

In conclusion, our results suggest that more than 90% of children and adolescents failing ART are predicted to benefit from TLD regimens. Given the low prevalence of K65R and accumulation of TAMs, genotyping is not a requirement before switching children and adolescents to TLD. However, given the challenges with regards to treatment adherence in this population, adequate virological monitoring is advised after treatment switch. Further studies are needed to investigate the clinical outcomes in children, adolescents and adults who switch to DTG in combination with NRTIs for which genotypic drug resistance testing predicts poor antiviral activity.

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