J Antimicrob Chemother 2019; **74**: 2030–2038
doi:10.1093/jac/dkz099 Advance Access publication 19 March 2019

Journal of Antimicrobial Chemotherapy

High predictive efficacy of integrase strand transfer inhibitors in perinatally HIV-1-infected African children in therapeutic failure of first- and second-line antiretroviral drug regimens recommended by the WHO

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Received 22 November 2018; returned 24 January 2019; revised 31 January 2019; accepted 7 February 2019

Objectives: The predictive efficacy of integrase (IN) strand transfer inhibitors (INSTIs) was investigated in HIV-infected children born to HIV-infected mothers in Africa.

Methods: Plasma was collected at the Complexe Pédiatrique of Bangui, Central African Republic, from INSTI-naive children (n=8) and adolescents (n=10) in virological failure (viral load >1000 copies/mL) after 5 years of first- and/or second-line combination ART (cART). IN, reverse transcriptase (RT) and protease (P) genes were genotyped and drug resistance mutations (DRMs) to INSTIs, NRTIs, NNRTIs and PIs were interpreted using the Stanford algorithm.

Results: Successful IN, RT and P genotypes were obtained for 18, 13 and 15 children (median age 11 years, range 5–18; 8 were female), respectively. Two (2/18; 11.1%) viruses from children treated with a first-line regimen had INSTI DRMs at codon 138 (E138K and E138T), which is known to harbour major resistance mutations, and also had the accessory mutations L74I, G140K, G140R and G163R. The majority (16/18; 88.9%) of HIV-1 IN sequences demonstrated full susceptibility to all major INSTIs with a high frequency of natural polymorphic mutations. Most (12/15; 80%) genotyped viruses harboured at least one major DRM conferring resistance to at least one of the WHO-recommended antiretroviral drugs (NNRTIs, NRTIs and PIs) prescribed in first- and second-line regimens.

Conclusions: INSTIs could be proposed in first-line regimens in the majority of African children or adolescents and may constitute relevant therapeutic alternatives as second- and third-line cART regimens in HIV-infected children and adolescents living in sub-Saharan Africa.

Introduction

Despite the encouraging enhancement in paediatric HIV care in sub-Saharan Africa, the widespread use of combination ART (cART) in the prevention of mother-to-child transmission (PTMCT) of HIV as well as in the care of HIV-infected children has unfortunately allowed the emergence of HIV strains highly resistant to the main antiretroviral (ARV) drugs, leading to high rates of virological failure.[1–3] Most studies that have evaluated the impact of HIV drug resistance mutations (DRMs) in HIV-1-infected children living in sub-Saharan Africa depict an alarming situation with high rates of accumulated pretreatment DRMs in infants born to HIV-infected mothers failing PTMCT and those born to untreated HIV-infected mothers.[3–8] Furthermore, the vast majority of HIV-infected African children failing NRTI, NNRTI and PI-based first- or second-line regimens show very worrying rates (up to 97%) of virological failure associated with MDR HIV strains accumulating high rates of DRMs.[6,9–18] As a consequence, the increasing number of DRMs to

the main ARV drugs prescribed in sub-Saharan Africa has considerably reduced the effectiveness of current paediatric regimens.[1,4,16] Thus, the paediatric therapeutic regimens currently recommended by the WHO may become no longer suitable in African settings, leading to a decrease in convenient therapeutic options in many sub-Saharan African countries.[15,16,18]

According to the 2016 revised WHO consolidated guidelines on the use of ARV, HIV-infected children failing PI-based first-line regimens could be switched to a second-line regimen containing an integrase strand transfer inhibitor (INSTI), and those failing a second-line regimen could be switched to a third-line regimen including new drugs with minimal risk of cross-resistance to previously used regimens, such as INSTIs.[19] INSTIs constitute a new class of ARV drugs, which may be used in both treatment-naive and treatment-experienced patients.[20] Three major INSTIs have been approved by the US FDA: the first-generation INSTIs raltegravir and elvitegravir, and the second-generation INSTI dolutegravir.[20] Globally, INSTIs achieve rapid and durable control of viral replication with minimal toxicity and have been shown to greatly improve paediatric outcomes in salvage regimens for children failing NRTI-, NNRTI- and PI-based first- and second-line regimens.[21–25] More recently, the new WHO interim guidelines updated in December 2018 recommend a dolutegravir-based regimen as the preferred first-line regimen in ART initiation for adolescents, and also for infants and children with approved dolutegravir dosing. In addition, a raltegravir-based regimen is now recommended as the preferred first-line regimen in ART initiation for neonates and as an alternative first-line regimen for infants and children for whom approved dolutegravir dosing is not available.[26] However, attention must be paid for adolescents and young adults as a recent analysis in childbearing-aged women in Botswana reported a possible association between exposures to dolutegravir at the time of conception and neural tube defects among infants.[27]

In the Central African Republic, HIV-1-infected children born to HIV-infected mothers attending the Complexe Pédiatrique of Bangui for care and treatment have a remarkably high prevalence of virological failure (around 60%) associated with very high rates of therapeutic failure and high rates of DRMs to NRTIs or NNRTIs (45%) and PIs (24%).[11,16,28–30] Overall, 55% of children receiving first-line therapy were eligible for a second-line regimen and 64% of children under a second-line regimen urgently needed third-line therapeutic options.[16]

Finally, the aim of the study was to investigate the frequency of DRMs and the prevalence of natural polymorphisms of the integrase gene (IN) in cART failure-experienced, INSTI-naive HIV-infected children living in Bangui, in order to estimate the predictive efficacy of INSTI-based paediatric regimens prior to their introduction in the country, as currently recommended for adolescents by the 2016 consolidated WHO guidelines for paediatric AIDS care in sub-Saharan Africa,[19,26] with further possible extension among children as young as 4 weeks old, including children receiving TB co-treatment.[26,31,32]

Patients and methods

Study design

The paediatric cohort of Bangui, Central African Republic, is an observational and prospective cohort of HIV-infected children who initiated cART between 2007 and 2009 and who were followed up at the Complexe Pédiatrique of Bangui for the treatment of paediatric AIDS, as previously described extensively.[11,16,28,30] Children attending the paediatric complex are mainly born to HIV-infected mothers who failed PTMCT.

For the present study, a random selection of one out of seven (14%) children from the cohort in virological failure according to the 2016 revised WHO threshold [viral load (VL) $\geq$3 log copies/mL or $\geq$1000 copies/mL][19] was carried out for IN sequencing. All selected children had been taking a first- or second-line WHO-recommended cART regimen for at least 6 months before inclusion.[19] None of the study children had ever received INSTIs.

Virological analysis

Plasma samples from selected children were obtained from the Complexe Pédiatrique, Bangui and brought in an ice pack to the virology laboratory of the Hôpital Européen Georges Pompidou, Paris, France. Genes for IN, reverse transcriptase (RT) and protease (P) were sequenced using the ViroSeq HIV-1 genotyping system (Celara Diagnostics, Alameda, CA, USA) with 1 mL of plasma sample and according to the manufacturer's instructions, as described previously.[11,16]

Genetic analysis and drug resistance

Mutations associated with resistance to NRTIs, NNRTIs, PIs and INSTIs were identified and interpreted using the Stanford University genotypic resistance interpretation algorithm, the HIV Drug Resistance Database (https://hivdb.stanford.edu/). The HIV-1 IN, RT and P sequences obtained from this study were uploaded to European Nucleotide Archive database with the accession number PRJEB29763. HIV-1 subtyping was established with IN sequences using the online genotyping tool of the NIH (https://www.ncbi.nlm.nih.gov/projects/genotyping/formpage.cgi). Phylogenetic analysis was carried out using MEGA 7 software (https://www.megasoftware.net).

Ethics statements

The study was formally approved by the Scientific Committee of Faculté des Sciences de la Santé of Bangui, which constitutes the national ethics committee in Central African Republic (reference #2UB/FACSS/CSVPR/09). Informed written consent was obtained from the mothers on behalf of the children participating in the study. The collected data were anonymized before the analyses.

Statistical analyses

Characteristics of the studied children and the results of this analysis were entered into a Microsoft Excel data sheet. Means are shown with the standard deviation (SD) and medians with the IQR.

Results

Study population

Eighteen [median age, 11 years; range, 5–18 years; 8 (44.4%) female; 10 adolescents (10–19 years of age) and 8 children (3 to <10 years, according to WHO classification[19])] of the 129 children and adolescents in virological failure from the Complexe Pédiatrique cohort were randomly selected. Socio-demographic and biological characteristics of the study children are summarized in Table 1. Most of the children (n=17, 94.4%) were on a first-line regimen for a mean duration of 6.2 years (range, 3.8–7.3 years). Fourteen of them received a combination of zidovudine (ZDV) + stavudine (d4T) + nevirapine (NVP), two children received

Table 1. Characteristics of ARV drug-experienced, INSTI-naive HIV-1-infected study children in virological failure followed up at the Complexe Pédiatrique of Bangui who were prospectively and randomly selected

Characteristic	Study children (n=18)
Age, years, median (range)	11 (5–19)
Sex, *n* (%)	
male	10 (55.6)
female	8 (44.4)
Therapeutic line, *n* (%)	
first-line	17 (94.4)
second-line	1 (5.6)
Treatment duration, years, mean ± SD (range)	6.2 ± 1.5 (3.8–7.3)
CD4 T cell count, cells/mm^3, mean ± SD (range)	674 ± 162.6 (55–2467)
Viral load, $\log_{10}$ copies/mL, mean ± SD (range)	4.3 ± 0.93 (3.2–6.3)
Resistance to ARV drugs[a]	
Total number of genotypes resistant to WHO-recommended drugs, *n* (%)[b]	12/15 (80)
DRMs to PI, *n* (%)	12/15 (80)
DRMs to NRTI, *n* (%)	11/13 (84.6)
DRMs to NNRTI, *n* (%)	12/13 (92.3)
DRMs to INSTI, *n* (%)	2/18 (11.1)
DRMs to NRTI and NNRTI, *n* (%)	11/13 (84.6)
DRMs to NRTI or NNRTI and PI, *n* (%)	9/15 (60.0)
DRMs to NRTI or NNRTI and INSTI, *n* (%)	2/18 (11.1)

[a]ARV resistance genotyping was carried out in 18 plasma samples from children with detectable plasma HIV-1 RNA VL; successful IN, RT and P genotypes were obtained for 18, 14 and 15 children, respectively.
[b]*n*, number of drug-resistance genotypes conferring resistance to one or more WHO-recommended drugs; the percentage indicates the ratio of the number of drug-resistance genotypes conferring resistance to one or more WHO-recommended drugs out of the total number of successful genotypes for the P, RT or IN gene.

ZDV + lamivudine (3TC) + efavirenz (EFV) and one child received a PI-based combination composed of d4T + 3TC + lopinavir boosted by ritonavir (LPV/r). Only one study child was under a second-line regimen consisting of d4T/3TC/LPV/r for a duration of 3.3 years after having received a first-line combination of ZDV/d4T/NVP for 1.3 years. Finally, at the time of sampling, the mean lymphocyte CD4 count was 674 cells/mm^3 (range, 55–2467) and the mean VL was 4.3 $\log_{10}$ copies/mL (range, 3.2–6.3).

IN genotyping and HIV-1 subtyping

Successful genotypes of the IN gene were obtained for all children. All the HIV-1 strains isolated in these children belonged to non-B subtypes, with a majority of CRF11_cpx (38.8%), subtype A (22.2%), CRF01_AE (16.6%), CRF25_cpx (11.2%) and subtype H and CRF02_AG (5.5%) (Figure 1).

Genotypic resistance in IN gene

The nucleotide sequence of the IN gene was available for 18 plasma samples and the distribution of detected mutations, including DRMs and polymorphisms, is depicted in Figure 2a.

Two (2/18; 11.1%) viruses from children under a first-line regimen had INSTI DRMs at codon 138, known to harbour major resistance mutations (Table 2). One HIV-1 strain showed the DRM E138K and the other showed E138T, which are both associated with potential low-level resistance (mutation score, 10) to dolutegravir and low-level resistance (mutation score, 15) to raltegravir and elvitegravir according to the Stanford University algorithm (Table 2). In addition, the HIV-1 strain harbouring the E138K DRM also displayed accessory mutations G140K, G163R (Table 2) and APOBEC-related mutations not associated with resistance, including G82E, E85K, G106K, D116N, D167N and E170K. Another HIV-1 strain displayed the accessory mutations G140R and L74I, and three APOBEC-related mutations: G70R, G149R and G247R. The following unusual mutations were also found in an HIV-1 strain: L234P, P238G, K240E, G247E, A248G and D256G. Finally, the unusual polymorphic mutation T112E was found in the HIV-1 strain harbouring the E138T DRM.

Polymorphic mutations in the IN gene were frequently observed: L101I/M, TT124A/G/N and T125A/P/V (100%) were the most represented, followed by G134D/N/S and L234I/P/V (94.4%); T112A/E/I/T/V (83.3%), D167E/N (77.7%), K136R/Q/T (66.7%), I72V (50.0%), S255D/G/N (38.9%), N222K/T, T206S and T218I/L/S (33.3%); and I135V, I208L and S119P (27.7%). Other polymorphic mutations were represented at <20% (Figure 2a).

When comparing the occurrence of polymorphic mutations by HIV-1 strain according to circulating recombinant forms (CRFs) or HIV-1 subtypes, CRF01_AE (mean number of mutations per genotype 19, range 13–24) and CRF25_cpx (mean number of mutations per genotype 19, range 14–24) were the most polymorphic subtypes, followed by subtype A (mean number of mutations per genotype 17.2, range 12–25), CRF02_AG (15 mutations in a unique genotype studied), CRF11_cpx (mean number of mutations per genotype 12.3, range 8–16) and finally the strain identified as a subtype H (12 polymorphic mutations).

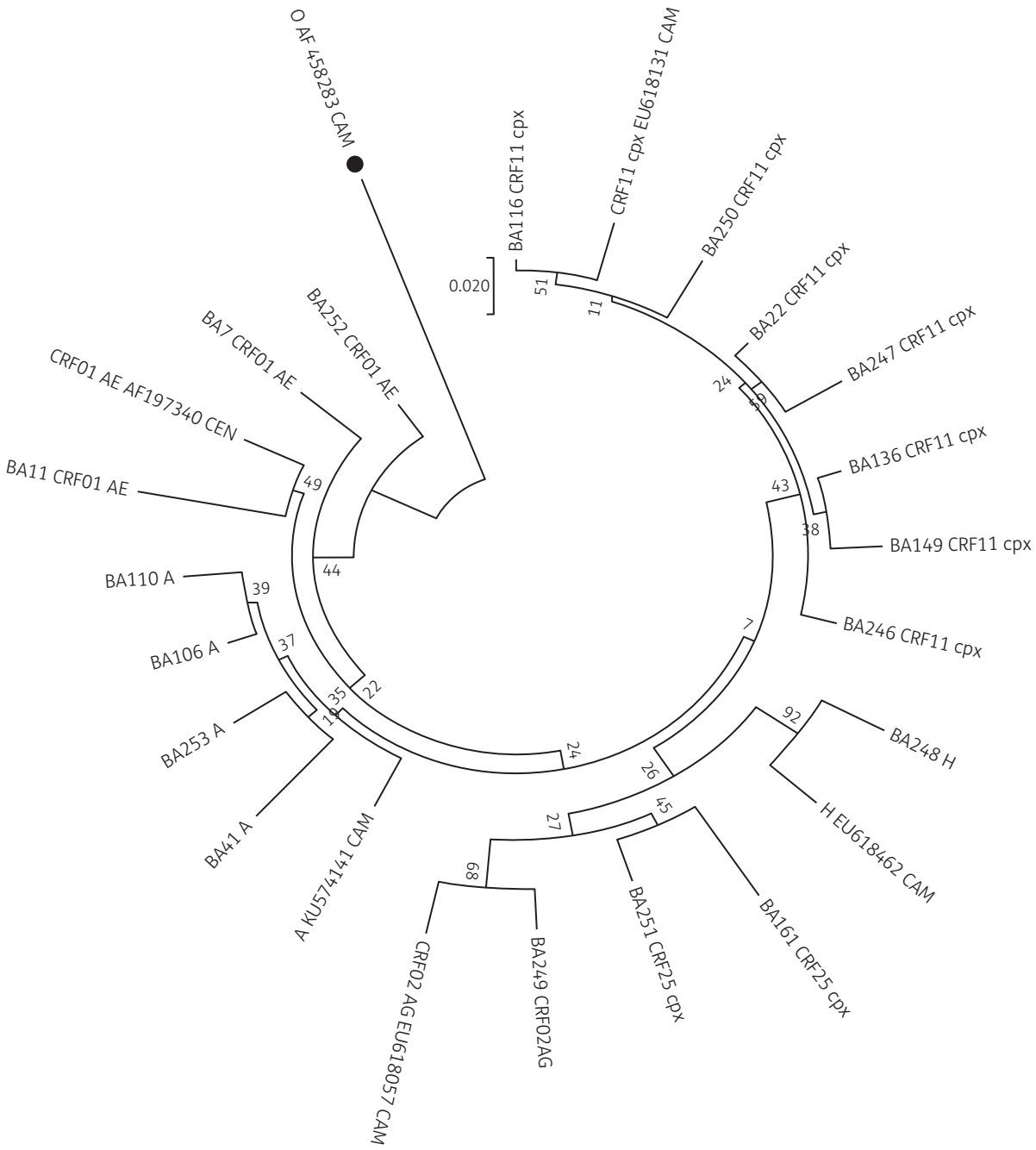

Figure 1. Molecular phylogenetic analysis of the genetic diversity of the HIV-1 IN gene from 18 children in Central African Republic. The genetic diversity (HIV-1 subtypes) of the 18 HIV-1 strains was assessed using the IN sequences. The molecular phylogenetic analysis was inferred by using the maximum likelihood method, with 1000 bootstrap replicates. The phylogenetic tree contains 18 HIV-1 integrase sequences from the study children and 6 sequences corresponding to 6 different HIV-1 subtypes retrieved from HIV-1 Los Alamos National Library database (HIV-1 LANL) (https://www.hiv.lanl.gov/content/sequence/HIV/mainpage.html). HIV-1 IN sequences from the study children are indicated by the two uppercase characters 'BA' followed by the inclusion rank number of the children, a space and the corresponding HIV-1 subtype. Each HIV reference sequence is labelled with its corresponding subtype followed by the GenBank accession number and the three characters (in uppercase) indicating the country where it was isolated. The tree is rooted with an HIV-1 subtype O sequence from Cameroon marked with a black circle. A and H are subtypes of HIV-1. CRF, circulating recombinant form; CAM, Cameroon; CEN, Central African Republic.

Taken together, the majority (16/18; 88.9%) of HIV-1 IN sequences demonstrated full susceptibility to all three major INSTIs with a large frequency of natural polymorphic mutations.

Genotypic resistance in RT and P genes

A total of 13 and 15 of the 18 selected plasma samples were successfully genotyped for RT and P genes, respectively. Most (12/15;

Table 2. Usual and unusual amino acid substitutions at drug resistance positions in the IN gene of the HIV-1 genome

	Amino acid drug resistance position																						
Drug resistance substitutions selected by INSTI	66[a]	74[b]	92[a]	97[b]	118[a]	121[a]	138[a]	140[b]	143[a]	145[a]	147[a]	148[a]	151[b]	153[b]	155[a]	157[b]	163[b]	263[a]
	T→A/I/K	L→I/M	E→Q	T→A	G→R	F→Y	E→A/K/T	G→A/C/S	Y→A/C/G/H/R/S	P→S	S→G	Q→E/G/H/K/R	V→L	S→Y	N→H/S/T	E→Q	G→R/K	R→K
Specimen (HIV-1 subtype)	Identified substitutions																	
BA11 (CRF01_AE)	–	–	–	–	–	–	E→K	G→K[c]	–	–	–	–	–	–	–	–	G→R	–
BA250 (CRF11_cpx)	–	–	–	–	–	–	E→T[d]	–	–	–	–	–	–	–	–	–	–	–
BA161 (CRF25_cpx)	–	L→I	–	–	–	–	–	G→R[c]	–	–	–	–	–	–	–	–	–	–

Dashes represent lack of mutation at a given position.
NA, not attributable.
[a]Major primary resistance mutation position.
[b]Accessory resistance mutation position.
[c]Unusual amino acid substitution.
[d]Rare amino acid substitution.

80%) genotyped viruses harboured at least one major DRM conferring resistance to at least one of the WHO-recommended antiretroviral drugs prescribed in first- and second-line regimens (Table 1). The distribution of DRMs and the polymorphism in RT and P genes are depicted in the Figure 2. DRMs to PIs corresponded mainly to natural polymorphism in the P gene while only the major DRMs V82A/F (n=3; 20%) and L33F (n=1, 6.7%) could be found. The majority of genotyped viruses harboured at least one mutation associated with NNRTI resistance (85.7%) or with NRTI resistance (78.5%). The genotypic predictive efficacy of NRTI and NNRTI drugs showed possible therapeutic options for study children (Table 3). At least one PI drug could be used for switching in all study children, at least one major NRTI could be used in all but one (92.8%) child, and at least one NNRTI could be used in the majority (78.6%) of children. Interestingly, concerning the best possible association with dolutegravir, all (100%) HIV-1 strains remained susceptible to darunavir, most (11/13; 84.6%) viruses to tenofovir and a minority to lamivudine (3/13; 23.1%).

Discussion

We herein report for the first time DRMs and polymorphism in the IN gene of HIV-1 strains from cART-experienced INSTI-naive HIV-1-infected children and adolescents living in Bangui. Nearly 90% of HIV-1 strains showed predicted full susceptibility to the three current major INSTI drugs, in association with a large proportion of natural polymorphic mutations. Only two (11.1%) children harboured INSTI DRMs at codon 138 (E138K/T), known to usually display primary major INSTI-selected resistance mutations. Taken together, these observations demonstrated the high predicted efficacy of INSTIs as alternative ARV options that could optimize current paediatric regimens in sub-Saharan African settings.

Currently, INSTIs are still not available for the treatment of HIV-infected children in sub-Saharan Africa, while the effectiveness of the current first- and second-line WHO-recommended paediatric regimens appears to be rapidly waning.[15,16,18] Data on the efficacy of INSTIs in African children are scarce. Almost 90% (88.9%) of HIV-1 strains from our study children never treated with INSTIs remain fully susceptible to all three current INSTIs, with a large proportion of natural polymorphic mutations. Two (11.1%) children harboured INSTI DRMs at codon 138 (E138K/T), known to usually display primary major INSTI-selected resistance mutations according to the Stanford University algorithm.[33] These findings are in keeping with previous reports indicating that major INSTI-selected DRMs are uncommon in INSTI-naive adult patients living in sub-Saharan Africa.[20,34–37] However, although these mutational events remain rare, they can nonetheless occur in INSTI-naive patients, as observed in our series. Similar rates of INSTI DRMs have also been reported in previous studies in INSTI-naive adult patients from other Central African countries harbouring, as in our study, a large proportion of HIV-1 non-B subtypes.[38,39]

In this study, one child exhibited the DRM E138K, conferring potential low-level resistance to dolutegravir and intermediate resistance to raltegravir and elvitegravir according to the Stanford University algorithm. Usually, E138K is a non-polymorphic mutation selected in the case of virological failure in patients receiving raltegravir, elvitegravir or dolutegravir.[40]

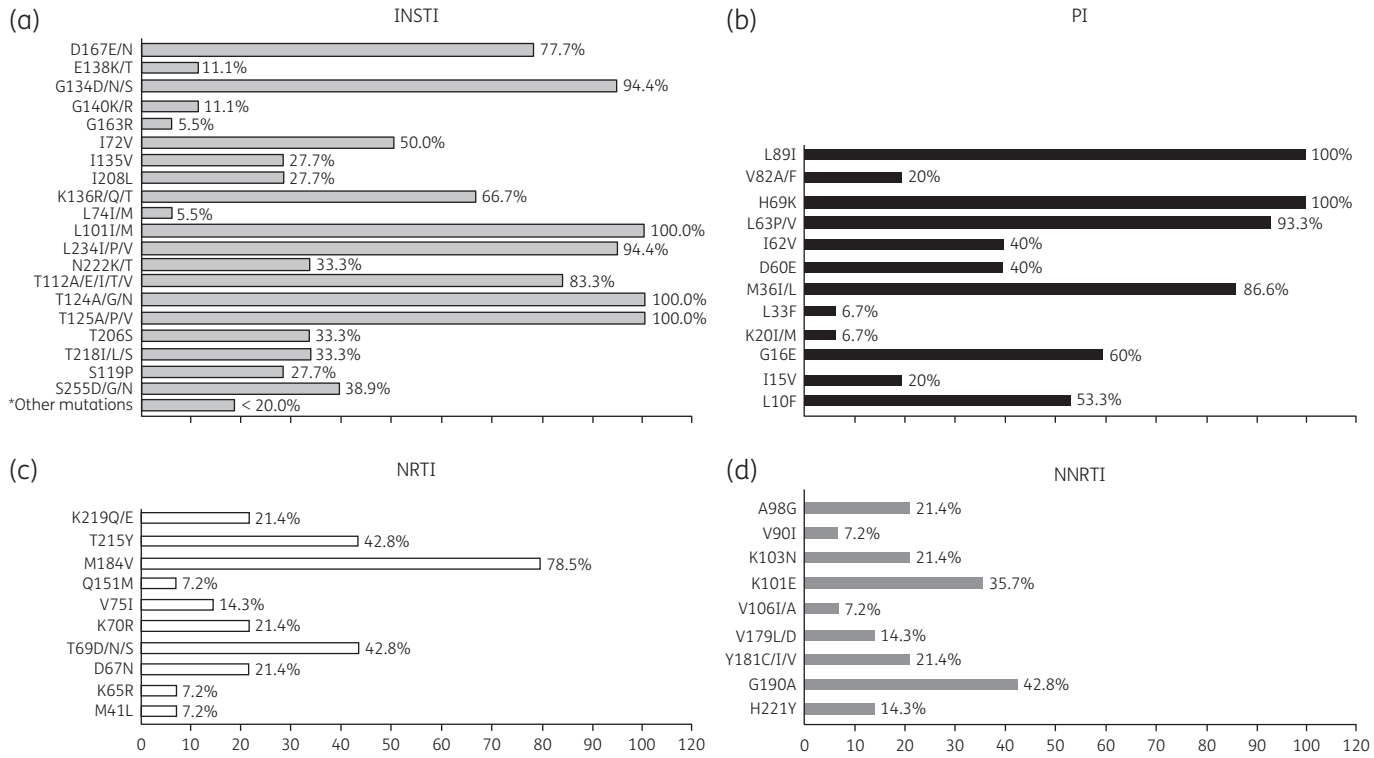

Figure 2. DRMs expressed as percentage observed in 14, 15 and 18 successful genotypes in HIV-1 RT, P and IN genes, respectively, obtained from 18 children in virological failure (HIV-1 RNA load >1000 copies/mL) followed up at the Complexe Pédiatrique of Bangui. (a) DRMs to INSTIs; (b) DRMs to PIs; (c) DRMs to NRTIs; and (d) DRMs to NNRTIs. The asterisk indicates polymorphic mutations occurring at <20% in the integrase gene [E96D, I203M, N254K and V260I (16.6%), A265V, D232N, D270H, G106A/K, K219N, R269K and V165I (11.1%), A23V, D116N, D229E, D253H, D279N, E85K, E157K, E170K, E198D/E, E212L, F181L, G70R, G82E, G136R, G149R, G247E, I60M, I200L, I220L, I268I/L, K14R, K111T, K173R, K186R, K188R, K236Q, K240E, M154I, P238G, Q221S/T, R107K, R166K, R224Q, S195T, S283G, V31I, V110I and Y227F (5.5%)].

But this DRM has also been described as part of the natural polymorphism of the IN gene.[41] According to the Stanford University genotypic resistance interpretation algorithm, this mutation alone does not significantly reduce INSTI susceptibility, but when it occurs in combination with other primary major INSTI-selected DRMs it is associated with high-level resistance to raltegravir and elvitegravir and intermediate reductions in dolutegravir susceptibility.[33] The other mutation at codon 138 observed in our study was E138T, conferring potential low-level resistance to dolutegravir and low-level resistance to raltegravir and elvitegravir according to the Stanford University algorithm. Contrary to our observations, E138T has been previously described as a rare non-polymorphic INSTI-selected mutation.[42] Our findings suggest that amino acid variation at the major resistance position 138 could also occur by other mutational pathways, such as natural polymorphism during viral replication or selective immune pressure, and not only by the selective drug pressure exerted by the INSTI-based treatment. Indeed, in our study we analysed HIV-1 strains isolated from INSTI-naive children, thus excluding the effect of INSTI drugs on the selection process of these mutations. Furthermore, previous studies evaluating the variability of the IN gene revealed that amino acid variations at codon 138 (E138A/D/K/T) could arise from G-to-A hypermutation resulting from APOBEC-mediated RNA editing,[42] and also from natural polymorphism during viral replication.[41,43] Further *in vivo* and *in vitro* studies are thus needed to better understand the clinical significance of these naturally occurring unusual DRMs in INSTI-naive children.

Along with the DRMs displayed at codon 138, the polymorphic accessory mutations G140K, G140R, G163R and L74I (5.5% for each) in the IN gene were also observed in our study. Although L74I and G163R are usually selected by INSTI drugs,[42,44] they have also been reported in INSTI-naive patients at rates similar to those reported in the present study;[39,40,42,44,45] alone, they do not appear to be associated with reduced INSTI susceptibility.[42–44] Concerning the variation at codon 140, the mutations G140K and G140R appear to be unusual mutations associated with polymorphism. Indeed, at position 140, the usual INSTI-selected accessory mutations are G140A/C/S, which are associated with a 3- to 5-fold reduction in susceptibility to elvitegravir when they occur alone.[46] In combination with primary major DRMs, they are associated with >100-fold reduction in susceptibility to elvitegravir and raltegravir and up to 10-fold reduction in susceptibility to dolutegravir.[47,48] However, polymorphism at position 140, similar to that observed in our study (G140K/R), has been described as leading to a higher genetic barrier for non-B subtypes to acquire the usual accessory INSTI-selected DRMs G140A/C/S at this position.[39,49] Consequently, the HIV-1 strains carrying these unusual polymorphic mutations (G140K/R) would develop less cross-resistance to different classes of INSTI drugs compared with HIV-1 strains that do not harbour these polymorphic mutations.

Table 3. Antiretroviral drug susceptibility in NRTIs, NNRTIs and PIs among ARV drug-experienced, INSTI-naive HIV-1-infected study children in virological failure followed up at the Complexe Pédiatrique of Bangui and prospectively included

	PI[a]				NRTI							NNRTI first generation		NNRTI second generation		
Specimen	DRV	ATV	SQV	TPV	3TC	ABC	ZDV	d4T	ddI	FTC	TDF	EFV	NVP	ETV	RPV	Drugs remaining efficient
BA7	S	S	R	S	R	S	R	R	S	R	S	R	R	S	S	DRV, ATV, TPV, ABC, ddI, TDF, ETV, RPV
BA11	S	S	S	S	R	S	R	S	S	R	S	R	R	I	S	DRV, ATV, SQV, TPV, ABC, d4T, ddI, TDF, RPV
BA22	S	S	S	R	R	S	I	I	S	R	S	R	R	R	R	DRV, ATV, SQV, ABC, ddI, TDF
BA41	S	S	S	S	R	S	S	S	S	R	S	R	R	S	S	DRV, ATV, SQV, TPV, ABC, ZDV, d4T, ddI, TDF, ETV, RPV
BA106	S	S	S	R	R	S	S	S	S	R	S	R	R	S	S	DRV, ATV, SQV, ABC, ZDV, d4T, ddI, TDF, ETV, RPV
BA110	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	DRV, ATV, SQV, 3TC ABC, ZDV, d4T, ddI, FTC, TDF, EFV, NVP, ETV, RPV
BA116	S	S	S	R	R	S	S	S	S	R	S	R	R	S	S	DRV, ATV, SQV, ABC, ZDV, d4T, ddI, TDF, ETV, RPV
BA149	S	S	S	S	R	S	S	S	S	R	S	R	R	S	S	DRV, ATV, SQV, TPV, ABC, ZDV, d4T, ddI, TDF, ETV, RPV
BA246	S	S	S	R	R	R	R	R	S	R	I	R	R	R	R	DRV, ATV, SQV, ddI
BA247	S	S	S	R	R	S	R	R	S	R	S	R	R	S	R	DRV, ATV, SQV, ABC, ddI, TDF, ETV
BA248	S	S	S	R	–	–	–	–	–	–	–	–	–	–	–	DRV, ATV, SQV
BA249	S	S	S	R	R	R	R	R	R	R	R	R	R	R	R	DRV, ATV, SQV
BA250	S	R	S	R	S	S	S	S	S	S	S	S	R	S	S	DRV, SQV, 3TC ABC, ZDV, d4T, ddI, FTC, TDF, EFV, ETV, RPV
BA252	S	S	S	R	–	–	–	–	–	–	–	–	–	–	–	DRV, ATV, SQV
BA253	S	R	S	R	S	S	S	S	S	S	S	S	S	S	S	DRV, SQV, 3TC ABC, ZDV, d4T, ddI, FTC, TDF, EFV, NVP, ETV, RPV

Dashes represent lack of available genotyped sequences.
S, susceptible; I, intermediate; R, resistant; ATV, atazanavir; DRV, darunavir; FPV, fosamprenavir; IDV, indinavir; LPV/r, lopinavir boosted by ritonavir; NFV, nelfinavir; SQV, saquinavir; TPV, tipranavir; 3TC, lamivudine; ABC, abacavir; ZDV, zidovudine; d4T, stavudine; ddI, didanosine; FTC, emtricitabine; TDF, tenofovir; EFV, efavirenz; NVP, nevirapine; ETV, etravirine; RPV, rilpivirine.
[a]All genotyped viruses were susceptible to the following PIs not included in Table 2: FPV, IDV, LPV/r and NFV.

In order to improve therapeutic care of children who failed the traditional first- and second-line cART regimens, a good alternative could be the combination of NRTI, NNRTI and PI drugs remaining active for these children, associated with an INSTI molecule with a high genetic barrier such as dolutegravir.[25,32,50,51] Indeed, second-generation dolutegravir has been demonstrated to have a high genetic barrier, thus minimizing the emergence of cross-resistance with the first-generation INSTIs and the other classes of ARV, making this drug the best option for a third-line salvage regimen for MDR HIV variants.[20–22,24,25,52,53]

A recent report from the WHO emphasized the introduction of a fixed-dose combination (FDC) of tenofovir, lamivudine and dolutegravir as a suitable optimized cART regimen in low- and middle-income countries.[32] In our study, more than three-quarters of the children (11/13; 84.6%) were resistant to lamivudine and the first-generation NNRTIs efavirenz and nevirapine (12/13; 92.3%), making these ARV drugs no longer suitable for a possible association as an FDC with dolutegravir. However, tenofovir remained fully efficient for most of the children (11/13; 84.6%), agreeing with the WHO report for its use in association with dolutegravir in an FDC-based regimen. Otherwise, in our study we found that a large proportion (9/13; 69.2%) of the HIV-1 strains remained susceptible to second-generation rilpivirine, which has also been described as a good candidate for an optimized dolutegravir-based treatment,[50,51] although the presence of the K103N mutation could limit the efficiency of rilpivirine.[54] Finally, PI drugs could also constitute an efficient option for these children and adolescents, as most of these ARV drugs, especially darunavir, remained fully efficient (15/15; 100%). Indeed, the use of dolutegravir in combination with darunavir in cART-experienced HIV-infected patients has been demonstrated to be convenient in switch therapy.[55,56] However, the cobicistat-boosted darunavir (darunavir/c) formulation should be preferred to ritonavir-boosted darunavir (darunavir/r), as darunavir/r reduces the plasma concentrations of dolutegravir when prescribed in combination,[57] unlike darunavir/c, which has very minimal impact on dolutegravir plasma concentrations.[58]

Our study has some limitations. The small sample size of included children may have introduced a selection bias. In addition, HIV-infected children living in Bangui frequently have a past history of sustained stavudine use in their ARV treatment, although this molecule has not been recommended in ART regimens since 2013.[59]

In conclusion, our observations demonstrate that INSTI drugs could be proposed in first-line regimens in the majority of children and adolescents, especially dolutegravir, which is currently recommended by WHO for adults, adolescents and children with approved dolutegravir dosing.[19,26] In addition, INSTIs may also constitute relevant therapeutic alternatives in HIV-infected African children and adolescents in therapeutic failure for first- or second-line WHO-recommended cART regimens. RT, P and IN genotypic backgrounds appear critical for selecting the most effective NRTI, NNRTI and PI drugs

before switching cART to an optimized combination along with dolutegravir in HIV-infected children in therapeutic failure.

Acknowledgements

We are particularly grateful to the staff of the Complexe Pédiatrique of Bangui in Central African Republic and to GIP-ESTHER (Ensemble pour une Solidarité Thérapeutique en Réseau, which is currently named Expertise France Santé), Paris, France.

Funding

The study was supported by internal funding.

Transparency declarations

None to declare.

Author contributions

Ralph-Sydney Mboumba Bouassa, Gérard Grésenguet, Charlotte Charpentier and Laurent Bélec conceptualized the study. Jean-Chrysostome Gody and Christian Diamant Mossoro-Kpinde recruited the patients. Ralph-Sydney Mboumba Bouassa, David Veyer, Matthieu Matta and Leman Robin performed the molecular analyses. Ralph-Sydney Mboumba Bouassa, Hélène Péré, Laurent Bélec and Charlotte Charpentier drafted the manuscript. All authors reviewed the manuscript and approved the final version.

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