Medicine®

OPEN

Escalating and sustained immunovirological dissociation among antiretroviral drug-experienced perinatally human immunodeficiency virus-1-infected children and adolescents living in the Central African Republic
A STROBE-compliant study

Christian Diamant Mossoro-Kpinde, MD, PhD, MSc[a,b,*], Jean-Chrysostome Gody, MD[b,c],
Ralph-Sydney Mboumba Bouassa, PhD, MSc[d,e], Sandrine Moussa, MSc[f], Mohammad-Ali Jenabian, PhD[g],
Hélène Péré, PharmD, PhD[e], Charlotte Charpentier, PharmD, PhD[h], Mathieu Matta, PharmD[e],
Jean De Dieu Longo, MD, PhD[b,i], Gérard Grésenguet, MD, PhD[b,i], Joël Fleury Djoba Siawaya, MD, PhD[j],
Laurent Bélec, MD, PhD, MPH, MSc[e]

Abstract

Sub-Saharan Africa has the vast majority (~90%) of new pediatric acquired immunodeficiency syndrome cases worldwide. Biologically monitoring HIV-infected pediatric populations remains challenging. The differential interest of human immunodeficiency virus (HIV)-1 RNA loads and CD4 T-cell counts is debated for the treatment of pediatric acquired immunodeficiency syndrome patients.

Long-term antiretroviral treatment (ART) outcomes regarding immunological and virological surrogate markers were longitudinally evaluated between 2009 and 2014 (over 57 months) in 245 perinatally HIV-1-infected children and adolescents born from HIV-infected mothers, treated at inclusion for at least 6 months by the World Health Organization-recommended ART in Bangui, Central African Republic.

Patients were monitored over time biologically for CD4 T-cell counts, HIV-1 RNA loads, and drug resistance mutation genotyping.

Children lost to follow-up totaled 6%. Four categories of immunovirological responses to ART were observed. At baseline, therapeutic success with sustained immunological and virological responses was observed in 80 (32.6%) children; immunological and virologic nonresponses occurred in 32 (13.0%) children; finally, the majority (133; 54.2%) of the remaining children showed discordant immunovirological responses. Among them, 33 (13.4%) children showed rapid virological responses to ART with an undetectable viral load, whereas immunological responses remained absent after 6 months of treatment and increased progressively

Mossoro-Kpinde et al. Medicine (2020) 99:21

Medicine

over time in most of the cases, suggesting slow immunorestoration. Notably, nearly half of the children (40.8% at baseline and 48.2% at follow-up) harbored discordant immunovirological responses with a paradoxically high CD4 T-cell count and HIV-1 RNA load, which are always associated with high levels of drug resistance mutations. The latter category showed a significant increase over time, with a growth rate of 1.23% per year of follow-up.

Our STROBE-compliant study demonstrates the high heterogeneity of biological responses under ART in children with frequent passage from 1 category to another over time. Close biological evaluation with access to routine plasma HIV-1 RNA load monitoring is crucial for adapting the complex outcomes of ART in HIV-infected children born from infected mothers.

Abbreviations: AIDS = acquired immunodeficiency syndrome, ART = antiretroviral treatment, d4T = stavudine, DRM = drug résistance mutation, I+ = successful immunological responder to therapy, I− = immunological nonresponder to therapy, NNRTI = nonnucleosidic reverse transcriptase inhibitor, NRTI = nucleosidic reverse transcriptase inhibitor, PI = protease inhibitor, V− = virological nonresponder, V+ = virological responder, VF− = presence of virological failure, VF+ = lack of virological failure, WHO = World Health Organization.

Keywords: adolescents, CD4 T cell count, Central Africa, children, human immunodeficiency virus-1, immunovirological dissociation, pediatrics, plasma HIV-1 RNA load, virological failure, World Health Organization recommendations

1. Introduction

Perinatal human immunodeficiency virus type (HIV-1) infection remains one of the leading causes of child mortality in sub-Saharan Africa.[1,2] Indeed, infants infected perinatally have poor survival prognoses; without antiretroviral treatment (ART), more than half of them do not reach their second birthday.[3] Since 2000, child mortality associated with HIV in sub-Saharan Africa has been significantly reversed.[1] Globally, 1.8 million (1.3 million–2.4 million) children (aged <15 years) had HIV in 2017, mainly in sub-Saharan Africa (88.3%), and 328 deaths occurred daily.[1,4,5]

In addition to the significant increase in the life expectancy of HIV-1-infected children receiving ART in sub-Saharan Africa,[5] the prolonged use of antiretroviral drugs has raised the question of its long-term impact on children's quality of life.[6–10] Likewise, several studies have reported on the outcomes of antiretroviral therapy in African pediatric populations,[11] but few of them have concerned virological and immunological long-term outcomes.[12–25] Interestingly, the correlation between immunological and virological failures in pediatric cohorts has often appeared minimal,[16,17,21] suggesting that the surrogate markers — HIV-1 RNA loads and CD4 T-cell counts — provide complementary rather than exclusive information for adapting ART for children.

To evaluate the management of pediatric acquired immuno-deficiency syndrome (AIDS), an observational cohort of HIV-infected children has been followed up since 2007 at the Complexe Pédiatrique in Bangui, Central African Republic, the main health care clinic for HIV-infected children in the country,[26–28] where an HIV epidemic was once considered "out of control."[29,30] The challenge of biologically monitoring HIV-infected pediatric populations in sub-Saharan Africa, with concerns about the differential interest of HIV-1 RNA loads and CD4 T-cell counts in caring for pediatric AIDS patients, prompted us to assess for the first time long-term prospective immunological and virological outcomes in a cohort of HIV-1-infected children attending the Complexe Pédiatrique and receiving an ART regimen adapted according to successive World Health Organization (WHO) guidelines.[31–35]

2. Material and methods

2.1. Study population

HIV-1-infected children followed up at the Complexe Pédiatrique in Bangui were prospectively recruited from May 2009 and followed up for 57 months until 2014 in a descriptive observational cohort study assessing their immunological and virological outcomes following ART. All the included children were born from HIV-1-infected mothers who were under ART for the prevention of mother-to-child transmission according to the national guidelines. Newborn children infected by HIV-1 despite prevention strategies were followed up and cared for according to the WHO recommendations for resource-limited countries.[31,32]

The inclusion criteria were as follows:

(1) having received ART for at least 6 months, consisting of first- or second-line regimens as recommended by WHO guide-lines[31–34];
(2) availability of simple demographic data on children (eg, age and gender) and treatment history (eg, duration of treatment and therapeutic line); and
(3) informed consent from each child's biological parent(s) or guardian(s).

The following definitions for children and adolescents were used according to the 2015 revised WHO recommendations[36]: A child is an individual between 1 and 10 years old, and an adolescent (ie, "teenager") is between 10 and 19 years old.

2.2. Plasma HIV-1 RNA loads and CD4 T-cell counts

Venipuncture Ethylene-diamine tetra-acetic acid blood samples were obtained from each included child both at inclusion and every 12 months during the follow-up period, according to the 2013 WHO recommendations.[34]

Plasma HIV-1 RNA load and CD4 T-cell measurements were carried out as previously described.[28] In brief, plasma HIV-1 RNA loads were measured at the Laboratoire National de Biologie Clinique et de Santé Publique in Bangui, using the Amplix platform developed by Biosynex (Strasbourg, France), which integrates a fully automated station for nucleic acid extraction (RNA and/or DNA) with a real-time polymerase chain reaction amplification station, using lyophilized Amplix HIV-1 RNA quantitative reagents (Biosynex). The assay detects HIV-1 groups M and O and several circulating recombinant forms.[37] The Laboratoire National de Biologie Clinique et de Santé Publique participates in an external quality assurance testing program organized by the virology laboratory of the Hôpital Européen Georges Pompidou in Paris, France. The CD4 T lymphocyte count was carried out using the Apogee auto 40 flow

Mossoro-Kpinde et al. Medicine (2020) 99:21

www.md-journal.com

cytometer from Apogee Flow Systems laboratories (Hemel Hempstead, London, England).

According to the 2013 WHO recommendations, the threshold for virological failure (VF+) was set at 1000 copies/mL.[34] This threshold was further consolidated by WHO in 2014[35] and 2016,[38] and that it continues to be used.[39]

Interlaboratory external quality control of the molecular and flow cytometry platforms was performed regularly using samples provided by the Hôpital Européen Georges Pompidou in Paris.[28,37,40,41]

2.3. Detection of drug resistance mutations (DRMs)

Detection of DRMs was carried out both at inclusion and after 39 months of follow-up (in 2013), as previously described.[28] In brief, aliquots of plasma were obtained at inclusion and follow-up, kept frozen at −80°C until being sent in a dry ice box to the virology unit of the Hôpital Européen Georges Pompidou in Paris, and then kept frozen at −80°C until their processing for resistance mutation genotyping. Antiretroviral resistance genotyping was performed on plasma specimens from children with a detectable HIV-1 RNA load. HIV-1 protease and reverse transcriptase pol genes were sequenced using the ViroSeq HIV-1 genotyping system (Celera Diagnostics, Alameda, CA). Resistance mutations were reported and interpreted based on the Agence Nationale de Recherches sur le SIDA et les Hépatites Virales algorithm (updated in September 2016; http://www.hivfrenchresistance.org). Protease and reverse transcriptase sequences were submitted to the European Nucleotide Archive with the following accession numbers: LT577626 to LT577673 and LT726745 to LT726792 (http://www.ebi.ac.uk/ena/data/view/). At baseline, antiretroviral resistance genotyping was carried out on the plasma samples of 125 patients with a detectable plasma HIV-1 RNA viral load. At follow-up, antiretroviral resistance genotyping was carried out on 58 plasma samples randomly selected from those of nearly half of 133 children with a detectable plasma HIV-1 RNA viral load; 50 plasma samples were obtained from children receiving first-line regimens, and 8 were from children receiving second-line regimens.

2.4. Classification according to immunovirological outcomes

A successful immunological response to therapy (I+) was defined as an increase in the CD4 T-cell count according to age at the follow-up visit (ie, CD4 T-cell count >750/µL for children younger than 5 years and CD4 T-cell count >500/µL for children and adolescents older than 5 years).[33,34] A virological response to treatment was defined as achieving a plasma HIV-1 RNA load below the limit of detection (ie, less than 20 copies/mL or 1.3 log copies/mL). Thus, virological responders (V+) were the children with an undetectable HIV-1 RNA load.

Finally, the study participants were classified into 4 categories according to their immunovirological responses: [I+, V+] for both immunological and virological responders, [I−, V+] for immunological nonresponders but virological responders, [I+, V−] for immunological responders but virological nonresponders, and [I−, V−] for both immunological and virological nonresponders.

2.5. Ethics statement

This study was formally approved by the Scientific Committee of the Faculté des Sciences de la Santé in Bangui, constituting the National Ethical Committee (Reference #2UB/FACSS/CSVPR/09) in the Central African Republic. Informed written consent was obtained from mothers for themselves and on behalf of their respective child participating in the study.

2.6. Statistical analyses

Paired patient data collected at inclusion and after the follow-up period were compared using the χ^2 test for categorical variables and unmatched Student t test for quantitative variables. Means and proportions were estimated with their 95% confidence intervals.

3. Results

Full descriptions of the pediatric cohort have been reported in 2009[27] and 2013.[28] The therapeutic regimen changed only slightly between 2009 and 2014, according to particular changes in successive WHO recommendations[31–35]: the progressive suppression of stavudine (d4T) and the introduction of tenofovir disoproxil fumarate.

3.1. Baseline characteristics of study children

At inclusion, 245 HIV-1-infected children were prospectively recruited within a period of 3 months (Table 1). The median age of the children was 9.1 years (range: 1–16 years), and the sex ratio (male/female) was 0.87 (114/131). All the children were already under ART with the majority (n=230; 93.9%) under a first-line ART regimen according to WHO recommendations,[31,32] for a mean duration of 18.6 months (range: 7.9–29.2 months). The remaining 15 (6.1%) children were under a second-line ART regimen for a mean duration of 31.2 months (range: 9.8–52.7 months). As recommended in the national guidelines and by the WHO in 2009,[31,32] at inclusion, the most prescribed treatment was the combination of d4T/lamivudine/nevirapine. In 2009, only a minority [18/245 (7.3%)] of the children were under a protease inhibitor (PI)-based regimen.

3.2. Baseline immunological and virological outcomes

Figure 1 depicts the classification of the 245 study children into 4 groups: [I+, V+], [I−, V+], [I+, V−], and [I−, V−], according to their immunovirological responses to treatment at baseline. Plasma HIV-1 viral loads were detectable (V−) in 132 study children (53.8%) but undetectable (V+) in 113 (46.2%) (Table 1). These 2 groups of children (V+ and V−) were each divided into 2 subgroups according to their immunological response as follows: The first subgroup [I+, V+] comprised 80 (32.6%) children who were immunological and virological responders with an undetectable viral load and a mean CD4 T-cell count estimated at 1063 cells/µL. The 32 children (13.0%) in the second subgroup [I−, V−] were immunological and virological nonresponders, with a very low mean CD4+ T-cell count (mean: 226 cells/µL) and high viral load (mean: 4.7 log copies/mL). More than half of the study cohort (133 [54.2%] children) showed immunological and virological discordant responses to treatment. Among them, 100 (40.9%) were immunological responders but virological nonresponders [I+, V−] with a high CD4 T-cell count (mean: 809 cells/µL) and high viral load (mean: 4.8 log copies/mL). The remaining 33 (13.5%) discordant children were immunological nonresponders but virological responders [I−, V+]

Mossoro-Kpinde et al. Medicine (2020) 99:21

Medicine

Clinicobiological characteristics of HIV-1-infected children followed up at the *Complexe Pédiatrique* in Bangui, at inclusion (n=245) and after 57 mo of immunovirological monitoring and therapeutic follow-up (n=220).

	Inclusion (n=245)				Follow up (n=220)			
	[I+, V+]	[I−, V+]	[I+, V−]	[I−, V−]	[I+, V+]	[I−, V+]	[I+, V−]	[I−, V−]
Number [N, (%)]	80 (32.6)*	33 (13.5)*	100 (40.9)*	32 (13.0)*	66 (30)†	21 (9.5)†	106 (48.2)†	27 (12.3)†
Age [yr, mean±σ (range)]	8.1±2.7 (1–17)	9.4±5.3 (1–18)	9.2±4.7 (1–18)	11.3±4.5 (2–17)	12.0±4.7 (4–18)	12.0±5.4 (4–18)	12.0±4.4 (4–18)	14.0±3.8 (6-18)
Sex [n, (%)]								
Male	30 (37.5)	17 (51.5)	52 (52.0)	15 (46.9)	29 (43.9)	10 (47.6)	52 (49.0)	10 (37.0)
Female	50 (62.5)	16 (48.5)	48 (48.0)	17 (53.1)	37 (52.1)	11 (52.4)	54 (51.0)	17 (63.0)
Therapeutic line [n, (%)]‡								
1st -line	79 (98.8)	30 (90.9)	91 (91.0)	30 (93.8)	62 (93.9)	17 (80.9)	103 (97.2)	16 (59.3)
2nd -line	1 (1.2)	3 (9.1)	9 (9.0)	2 (6.2)	4 (6.1)	4 (9.1)	3 (2.8)	11 (40.7)
Treatment duration [yr, mean±σ (range)]	1.3±0.6 (0.5–5.0)	0.7±0.3 (0.5–1.5)	1.9±0.9 (0.5–5.0)	2.3±1.3 (0.5–5.0)	6.6±8.7 (3.7–6.4)	11.3±15.3 (3.7–4.7)	6.7±10.8 (2.7–9.9)	21.12±21.72 (3.7–8.5)
Viral load [log copies/mL, mean±σ (range)]	NA	NA	4.8±1.5 (1.7–7.7)	4.7±1.7 (1.9–7.7)	NA	NA	4.6±1.3 (1.7–6.9)	4.3±0.9 (2.8–6.2)
CD4 T cell count [cells/µL, mean±σ (range)]	1062±551 (508–3121)	314±204 (11–678)	809±280 (347–1967)	225±153 (8–490)	982±366 (509–1985)	368±221 (24–731)	781±229 (498–1587)	250±135 (25–458)
Adherence [%, mean±σ, (range)]§	91.7±2.6 (85–95)	82.8±8.0 (65–95)	90.4±2.2 (85–95)	67.9±12.4 (50–90)	92.1±3.4 C (75–95)	87.8±7.5 (70–95)	90.1±1.8 (85–95)	69.2±7.2 (60–80)
Lost-of-follow-up [n/N, (%)]	NA	NA	NA	NA	4/80 (5)	3/33 (9)	3/100 (3)	15/32 (47)
Total number of resistance to WHO-recommended drugs [n, (%)]‖	NA	NA	86/94 (91)	29/31 (94)	NA	NA	40/40 (100)	17/18 (94.4)
DRMs to PI [n, (%)]	NA	NA	26 (27.6)	3 (9.6)	NA	NA	6 (15)	3 (16.6)
DRMs to NRTI [n, (%)]	NA	NA	47 (50.0)	13 (41.9)	NA	NA	18 (45.0)	7 (38.8)
DRMs to NNRTI [n, (%)]	NA	NA	50 (53.2)	16 (51.6)	NA	NA	17 (42.5)	9 (50)
DRMs to NRTI and NNRTI [n, (%)]	NA	NA	41 (43.6)	11 (35.5)	NA	NA	16 (40.0)	4 (22.22)
DRMs to NRTI or NNRTI and PI [n, (%)]	NA	NA	18 (19.1)	1 (3.2)	NA	NA	4 (10.0)	3 (16.6)

* Number of patients who were immunological responders or nonresponders and virological responders or nonresponders at baseline.

† Number of patients who were immunological responders or nonresponders and virological responders or nonresponders at follow-up.

‡ Number of children in first- or second-line regimens included in each of the 4 immunovirological subgroups at baseline and follow-up.

§ Adherence (Ad) was assessed as described previously,[26] using an empirical questionnaire addressed to the parent or child, according to the child's age, including the following variables: (1) α: number of pill(s) forgotten during the previous week; (2) β: number of pills taken inappropriately during the previous week; and (3) ɣ: number of days without drug intake during the previous week. Quantitative estimation of Ad was calculated as follows: $Ad = (1 − [(\alpha/14) + (\beta/7) + (ɣ/24)]/3) \times 100$. The variables α, β, and ɣ were rounded to the nearest integer. Finally, Ad was estimated as "very good" if Ad ≥ 90%, "good" if 80% < Ad < 90%, "middle" if 60% < Ad ≤ 80%, and "bad" if Ad ≤ 60%.

‖ Number of drug resistance genotypes harboring resistance to one or more WHO-recommended drugs. At baseline, antiretroviral resistance genotyping was carried out on plasma samples of 125 patients with a detectable plasma HIV-1 RNA viral load. At follow-up, antiretroviral resistance genotyping was carried out on 58 plasma samples randomly selected from those of nearly half of 133 children with a detectable plasma HIV-1 RNA viral load; and 50 plasma samples were obtained from children receiving first-line regimens and 8 were from children receiving second-line regimens.

σ = standard deviation, DRM = drug resistance mutation, HIV = human immunodeficiency virus, NA = not attributable, NNRTI = nonnucleosidic reverse transcriptase inhibitor, NRTI = nucleosidic reverse transcriptase inhibitor, PI = protease inhibitor, [I+,V+] = patients who were both immunological and virological responders, [I−,V+] = patients who were immunological nonresponders but virological responders, [I+,V−] = patients who were immunological responders but virological nonresponders, [I−,V−] = patients who were both immunological and virological nonresponders.

with a low CD4 T-cell count (mean: 314 cells/µL) and undetectable viral load.

Among the 125 successful genotypes, 94 were isolated from the immunological responders [I+, V−] and 31 from the immunological nonresponders [I−, V−] (Fig. 2).

In the subgroup of immunological responders [I+, V−], 91% of the children harbored viruses resistant to the WHO-recommended nucleosidic reverse transcriptase inhibitors (NRTIs) and/or non-NRTIs (NNRTIs) antiretroviral drugs. The mutation V82A, previously identified as being more frequent in patients with a favorable immunological response despite virological failure,[42] was observed in viruses from 5 (4.7%) children in the [I+, V−] group. All (100%) the children in this group harbored viruses with at least 1 resistance mutation to antiretroviral drugs.

In the subgroup of immunological nonresponders [I−, V−], 94% of the children harbored viruses with at least 1 resistance mutation to antiretroviral drugs (Fig. 2 and Table 1).

Overall, at baseline, about 10% (12/125) of the patients with a detectable viral load harbored viruses genotypically resistant to

the 3 molecules included in the main prescribed antiretroviral combination (d4T/lamivudine/nevirapine) (Fig. 3).

3.3. Longitudinal outcomes of study children after temporal follow-up

In 2014, d4T was progressively replaced with tenofovir disoproxil fumarate, and 12.7% (28/220) of children were under a PI-based regimen.

As depicted in Figure 1, 10% (25/245) of the study patients were lost to follow-up, whereas most (220/245; 90%) of the included children at baseline were followed up prospectively until 2014. Among them, 54% were girls (119/220) with a median age of 13.8 years (range: 5–≥18 years). The majority of the children and adolescents (n=198; 90%) received a first-line regimen according to the revised 2013 WHO recommendations,[34] for a mean duration of 65.6 months (range: 18.8–69.9 months) for first-line treatment. The remaining children and adolescents (n= 22; 10%) received a second-line regimen for a mean duration of 78.2 months (range: 13.3–88.3 months).
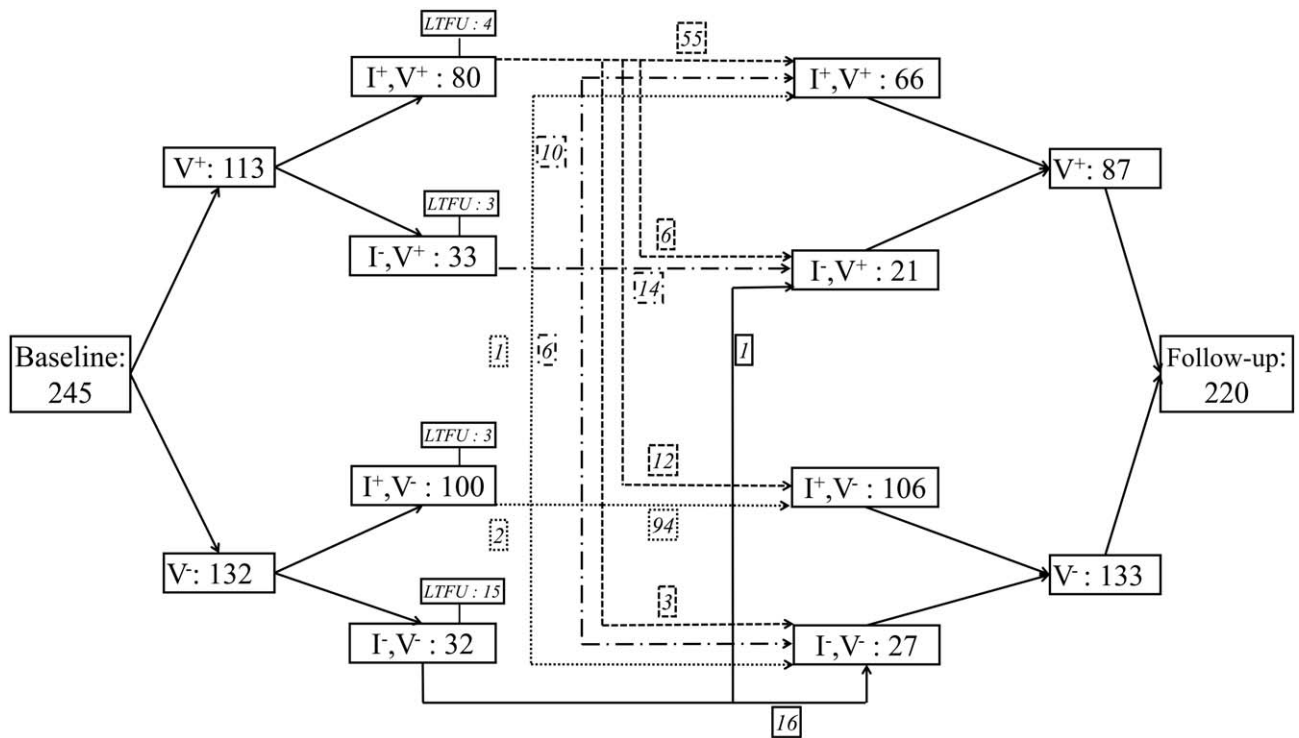
Figure 1. Longitudinal follow-up from 2009 to 2014 of 245 HIV-1-infected children born from HIV-positive mothers and treated by WHO-recommended regimens at the *Complexe Pédiatrique* in Bangui according to their immunovirological responses. HIV = human immunodeficiency virus, LTFU = lost to follow-up, WHO = World Health Organization.

Although none of the 4 subgroups of patients were spared, most of the children lost to follow-up (15/25; 60%) were in the group of immunological and virological nonresponders [I⁻, V⁻] (Fig. 1 and Table 1).

Plasma HIV-1 viral load measurements yielded high rates (60%) of virological nonresponders (V⁻), most of whom (96%) were in virological failure according to the WHO criteria (ie, circulating viral load above 1000 copies/mL) (Fig. 2). Long-term immunological responses to ART enabled refining the biological responses to ART. Thus, the longitudinal landscape of the children cohort remained over time almost the same as that at baseline, with 57.8% (127/220) of the patients showing discrepant responses to treatment ([I⁻, V⁺] and [I⁺, V⁻]), 30.0% (66/220) in therapeutic success ([I⁺, V⁺]), and 12.2% (27/220) with unfavorable immunovirological profiles ([I⁻, V⁻]) (Fig. 1 and Table 1).

Most of the immunological and virological responder children (55/76; 72.3%) at baseline consistently remained in full therapeutic success after 5 years of ART; only 3 (3.9%) of them experienced therapeutic failure after the follow-up period; and the remaining immunological and virological responder children at baseline (18/76; 23.7%) showed immunological and virological discrepant responses to treatment at follow-up: [I⁺, V⁺] → [I⁻, V⁺]: 6 (7.9%) and [I⁺, V⁺] → [I⁺, V⁻]: 12 (15.8%).

The majority (16/17; 94.1%) of the immunological and virological nonresponder children in therapeutic failure at baseline still failed to restore their CD4 count and viral load ([I⁻, V⁻] → [I⁻, V⁻]). Only 1 (5.8%) of them succeeded in controlling viral replication, but not in restoring the CD4 T-cell count ([I⁻, V⁻] → [I⁻, V⁺]).

Most of the immunological responders and virological nonresponders (94/97; 96.9%) remained in the same subgroup [I⁺, V⁻] during the longitudinal follow-up. Only 1 (1.0%) of them

succeeded in controlling viral replication ([I⁺, V⁻] → [I⁺, V⁺]), and the remaining 2 (2.0%) children in this subgroup experienced therapeutic failure with unfavorable immunovirological responses ([I⁺, V⁻] → [I⁻, V⁻]).

Regarding the discordant children [I⁻, V⁺] at baseline, 46.7% (14/30) of them remained in the same subgroup, 33.3% (10/30) evolved to immunological and virological responses with therapeutic success [I⁺, V⁺], and 20.0% (6/30) experienced therapeutic failure with unfavorable immunovirological responses ([I⁻, V⁺] → [I⁻, V⁻]).

After 39 months of follow-up, 96% (56/58) of the 58 genotypes from the child cohort with detectable viral loads (V⁻) and/or in virological failure according to the 2013 revised WHO criteria (V⁻ or VF⁺) harbored at least 1 DRM (DRM⁺) (Figs. 2 and 3). The distributions of DRMs in the virological nonresponders and in the patients in virological failure were similar, with a minority showing DRMs to PIs and around half of them exhibiting DRMs to NRTIs or NNRTIs. As expected, a high frequency of natural polymorphisms in the protease gene sequences was observed in over 90% of the sequenced viruses. Regarding the NRTI class, nearly half of the nonresponder patients (V⁻) displayed viruses that harbored at least 1 DRM associated with NRTI resistance (Table 1). At follow-up, most of the sequenced viruses remained susceptible to the majority of the NRTIs, NNRTIs, and PIs proposed for the WHO-recommended second-line regimen[33,34] (not shown).

3.4. Distribution of children showing discordant immunovirological responses [I⁺, V⁻] over time

In 2014, children showing discrepant immunological and virological responses to treatment represented 58% (127/220) of the cohort (Fig. 1 and Table 1).

Mossoro-Kpinde et al. Medicine (2020) 99:21

Medicine
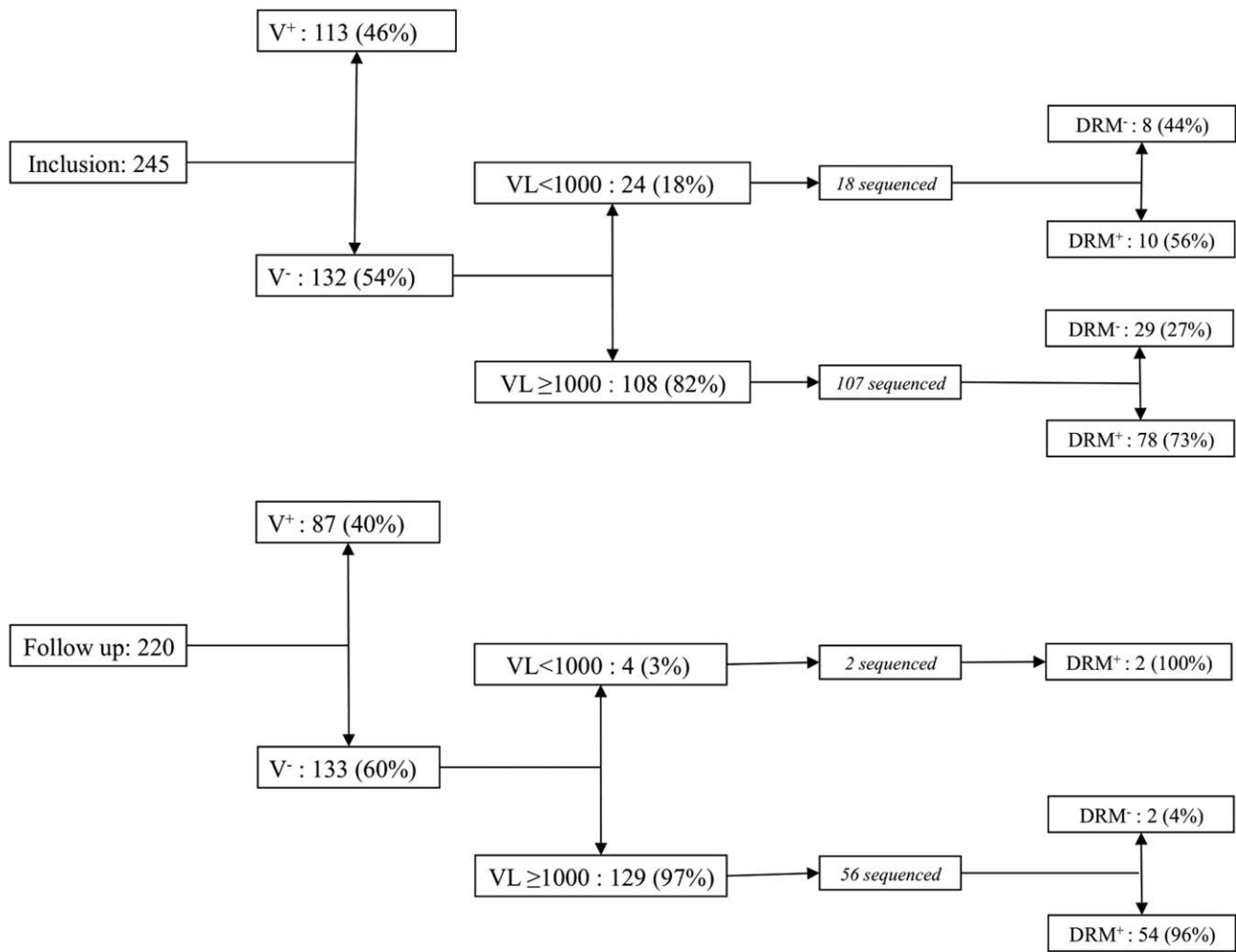
Figure 2. Flow diagram of molecular analyses for drug resistance mutation genotyping in study children harboring a detectable HIV-1 RNA load despite therapeutic treatment according to WHO recommendations, at baseline (n = 245) and after 57 mo of follow-up (n = 220). HIV = human immunodeficiency virus, WHO = World Health Organization.

A significant proportion of them (106/127; 83.5%) were immunological responders but virological nonresponders [I⁺, V⁻] with a high CD4 T-cell count (mean: 782 cells/μL; range: 552–1011 cells) and viral load (mean: 4.6 log copies/mL; range: 2.3–5.9 log copies).

The longitudinal follow-up of children showing virological failure but immunological responses [I⁺, V⁻] revealed a significant increase in this subgroup of children, from 40.9% (100/245) at baseline to 48.2% (106/220) after 57 months of follow-up with an annual growth rate of 1.23%.

At baseline, 91% (91/100) of the discordant [I⁺, V⁻] children were treated with a first-line regimen; after a mean duration of 76.7 months (range: 9.7–80.8 months) of therapeutic follow-up, most of them continued their first-line treatment, and only 3 (2.8%) of them were switched to second-line therapy (Table 1).

The majority (91%) of the sequenced viruses from discordant [I⁺, V⁻] children at baseline were resistant to WHO-recommended antiretroviral drugs (Fig. 2 and Table 1). All (100%) the sequenced viruses from discordant [I⁺, V⁻] children at follow-up harbored genotypic resistance. Although there was a slight decrease in the mean viral load of these patients during follow-up (4.8 log copies/mL at baseline to 4.6 after follow-up), this variation was not statistically significant ($P = .13$) (Fig. 4 and Table 1). Likewise, there was no statistically significant change in CD4 T-cell counts over time in the [I⁺, V⁻] discordant children ($P = .6$) (Fig. 4). Furthermore, 55 children (mean age: 11 years; range, 4–18 years) among the 94 children (mean age: 12 years; range, 4–18 years) remaining in the group [I⁺, V⁻] during the follow-up showed a significant decrease in their mean CD4 T-cells count overtime (916.1 cells/μL at baseline vs 784.7 cells/μL after follow-up [$P < .01$]) (Fig. 1), while their CD4 T-cells counts remained all above the thresholds of immunological failure according to age (ie, CD4 T-cell count >750/μL for children less than 5 years and >500/μL for children and adolescents older than 5 years).

There was also a slight decrease in the mean viral load of the [I⁻, V⁻] patients during follow-up, without statistical significance ($P = .31$) (Fig. 4).

4. Discussion

In this study, the long-term WHO-recommended ART was longitudinally evaluated using immunological and virological surrogate markers in 245 perinatally HIV-1-infected children and
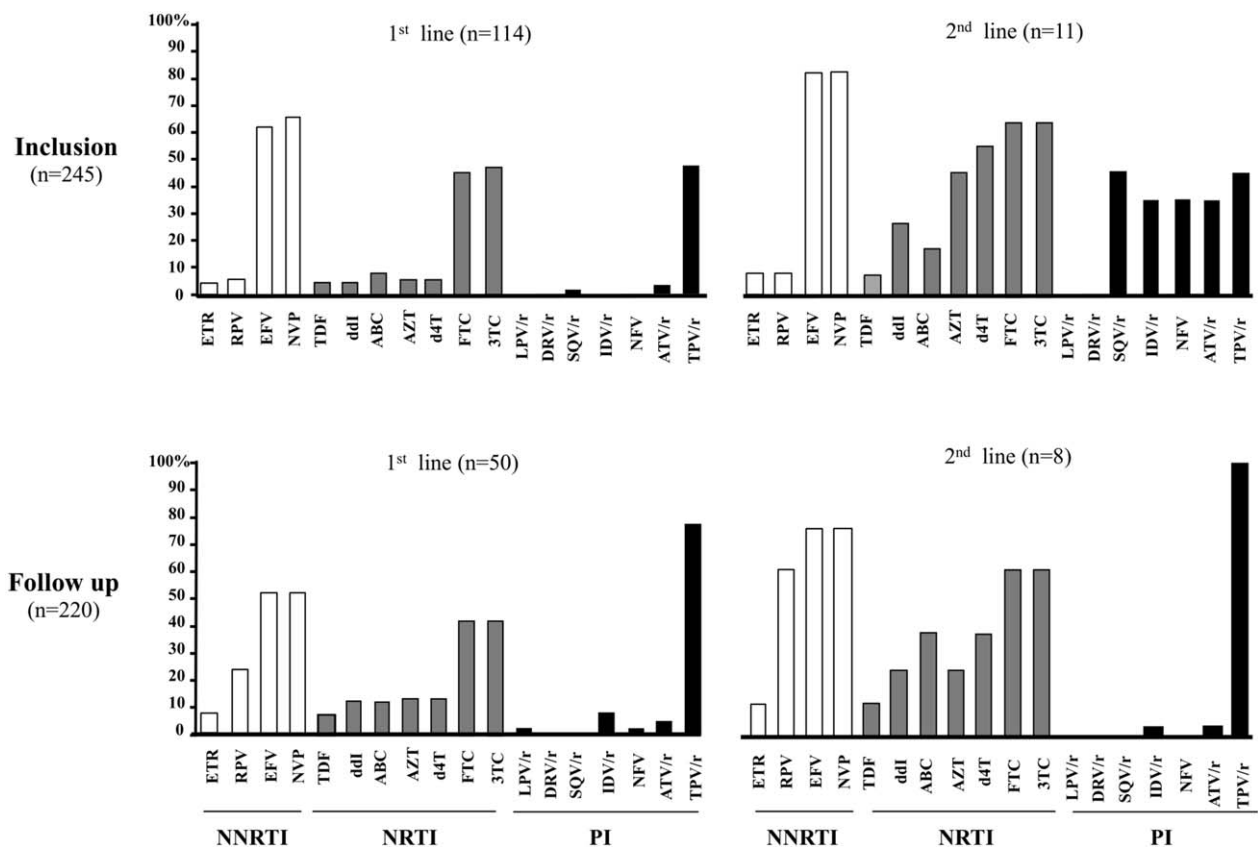
Figure 3. Percentage of genotypes harboring mutations conferring resistance to the major WHO-recommended antiretroviral drugs at baseline (n = 245) and after 39 mo of ART (n = 220) according to the therapeutic lines in HIV-1-infected children born from HIV-positive mothers and cared for at the *Complexe Pédiatrique* in Bangui. Genetic analysis of the 58 HIV-1 pol sequences obtained in a 2013 follow-up showed broad genetic diversity. Thus, most children were infected with CRF11_cpx (34.4%) and CRF01-AE (18.9%), or with HIV-1 subtype A (12.1%). Furthermore, a wide variety of HIV-1 subtypes were observed: 5.1% each for CFR02_AG, CRF13_cpx, H, D; and 3.4% each for CRF15 and subtypes F1 and B. Finally, with the lowest proportion (1.7%) subtypes C and G. ART = antiretroviral treatment, CRF = circulating recombinant form, HIV = human immunodeficiency virus, WHO = World Health Organization.

adolescents born from HIV-infected mothers and followed up for 57 months at the Complexe Pédiatrique in Bangui. Children lost to follow-up totaled only 6%, indicating a high retention rate. Strong heterogeneity was observed in the immunological and virological responses to ART with the identification of 4 categories of immunovirological responses: full therapeutic success with sustained immunological and virological responses to treatment in nearly one-third of children, full therapeutic failure with immunological and virological nonresponses in nearly one-tenth of children always associated with high levels of DRMs, and discordant immunovirological responses in the majority (∼60%) of the remaining children.

Immunological and virological responses [I+, V+] with restoration or maintenance of normal CD4 T-cell counts and suppression of plasma HIV-1 RNA loads were observed in one-third of the cohort children. Our findings are consistent with those of previous reports on the frequent effectiveness of ART in HIV-infected children living in resource-limited countries, mainly in sub-Saharan Africa.[20,43–49] These observations justify the necessity for biologically monitoring affected children and adolescents at least once a year, especially for assessing their HIV-1 RNA load, regardless of their CD4 T-cell count, to enhance treatment at the slightest suspicion of treatment failure.[33,34,39]

The group of children in full therapeutic failure [I−, V−], in whom the plasma HIV-1 RNA load was elevated over time and

the CD4 T-cell count remained persistently low after the follow-up period, represented slightly more than one-tenth of the cohort. We defined therapeutic failure according to the nonresponses of the 2 immunological and virological surrogate markers, which may explain the apparently low rate of treatment failure below the range of 19.7% to 53.0% in sub-Saharan Africa.[10,12,25,44,50–56] Furthermore, this group was strongly associated with a high rate of patients lost to follow-up (47%), which could indicate the final rate of therapeutic failure. The frequent absence of being switched to a second-line regimen, despite a persistent detectable viral load in the long-term first-line-treated study children, would have likely led to an increased rate of virological failure, thus reinforcing the group of full therapeutic failure.[10,11,15,56,57] Only 1 patient in the [I−, V−] group was moved to the discordant immunological nonresponders group [I−, V+] after the follow-up. Indeed, it is well documented that HIV-1 induces rapid depletion of CD4 T cells before ART initiation,[58–60] providing low CD4 T-cell count recovery in patients starting ART with severe immune depression.[61]

More than half (58%) of our cohort exhibited unexpected discrepant responses to ART after nearly 5 years of follow-up, indicating that the correlation between immunological and virological failures was minimal, as previously observed in African pediatric cohorts.[21,24] Discordant immunological and

Mossoro-Kpinde et al. Medicine (2020) 99:21

Medicine

CD4 T cell count

Viral load
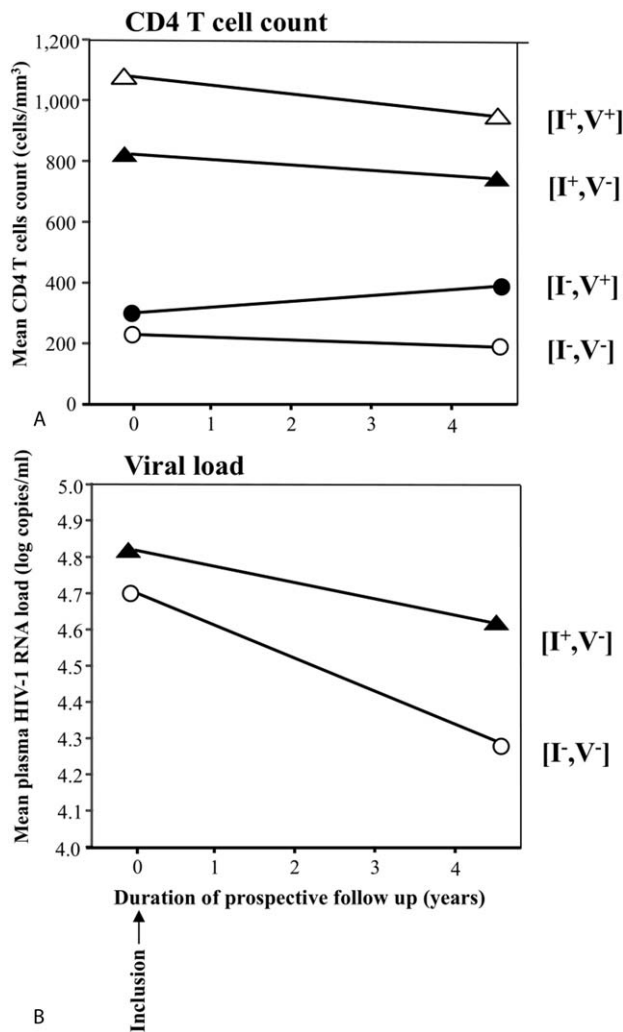
Figure 4. Variation over 57 mo of monitoring mean CD4 T-cell counts (A) and plasma HIV-1 RNA loads (B) according to the subgroups of immuno-virological responses to ART in HIV-1-infected children treated according to WHO recommendations. Discordant [I+, V−] children showed a decrease (Δ* = 0.2) in the mean HIV-1 RNA load, although the variation over time was not statistically significant (*P* = .13). ART = antiretroviral treatment, HIV = human immunodeficiency virus, WHO = World Health Organization.

tion, exhaustion, and apoptosis.[59,72–74] Thus, the extent of immune activation at the time of ART initiation can be associated with the development of discordant immune responses.[69,70] Long-term immunological nonresponder patients must be followed up with caution because persistent discordant immune responses may be closely associated with severe non-AIDS events[71,75] and with mortality risk in ART.[17,18,60,65,76] The consolidated 2013 WHO guidelines for treating HIV infection strongly recommended for the first time the use of HIV RNA load monitoring regardless of CD4 T-cell counts for routine identification of ART failure.[34] However, where appropriate, CD4 T-cell counts for patients under long-term ART remain crucial immunological indicators for identifying discordant immune responses.[77,78] The transition of nearly one-third (30%) of immunological nonresponders [I−, V+] to the group of patients who were therapeutically successful [I+, V+] was observed after the follow-up period. These findings suggest that these discordant children and adolescents were probably in immunorestoration and can thus be described as "slow progressors."[79]

Finally, among the children and adolescents who exhibited discordant responses to treatment after the follow-up period, 83.5% were classified as immunological responders but virological nonresponders [I+, V−]. Although these patients failed to control HIV-1 viral replication after the follow-up period and thus were all in virological failure with viruses harboring high rates of DRMs (100%), their CD4 T-cell counts remained persistently high, above normal levels. An increased CD4 T-cell count, higher than the WHO threshold for immunological success without suppressing viral loads that demonstrate discordant responses, has been reported.[38,60,80] The following hypotheses can be considered. First, the recovery of thymic function and high thymic output under ART favor the immunorestoration of HIV-1-infected children with a sustained CD4 T-cell increase, despite the persistence of viral replication.[66,81–84] Second, partial virus suppression under certain antiretroviral drug regimens (mainly PI) may reduce CD4 T-cell turnover and activation, thereby resulting in sustained CD4 T-cell gains, despite detectable viral replication.[61,85,86] However, only a minority of children in the [I+, V−] group in the study cohort received long-term ART containing PI. Third, about 5% of the children in the discordant [I+, V−] group harbored viruses exhibiting a resistance mutation (V82A in protease) previously reported in discordant patients.[42] Fourth, discordant responses during ART may be related to HIV-directed immune responses, diminished cellular activation, and preservation of non-syncytium-inducing viruses, as previously reported in adults.[80] Fifth, the accumulation of high levels of DRMs over time could have provided impaired viral fitness with lower viral replication capacities.[87–91] Furthermore, the longitudinal observation of our cohort children who were in virological failure but immunological responders [I+, V−] revealed a progressive and statistically significant increase over time, with a growth rate of 1.53% per year, suggesting selective advantages for the virus as well as for the discordant children, who appeared unexpectedly tolerant to the virus. Finally, our observations suggest that the discordant [I+, V−] group poses crucial concerns about therapeutic options, as previously discussed.[84] Maintaining such children in the current line certainly enables placing therapeutic pressure on potentially defective viruses or viruses with diminished fitness, which would lead despite resistance to a positive therapeutic effect.[80] Integrase strand transfer inhibitors

virological responses under ART have been reported for adults[62–65] and children[17,18,21] in Western countries[62–64] and resource-limited settings.[17,18,21,65] At inclusion, a small number of study children (13.0%) belonged to the group of immunological nonresponders but virological responders [I−, V+]. Despite good adherence to treatment and the control of HIV-1 viral replication, these children failed to restore normal CD4 T-cell counts despite long-term ART. CD4 T-cell alteration can begin before ART initiation due to direct effects of HIV on the thymus and depletion of progenitor cells.[58–60] Some studies have reported that thymic output may be disproportionally affected in patients who begin receiving ART at lower CD4 T-cell counts, leading to the under-reconstitution of naive CD4 T cells.[59,66–68] The exhaustion of the immune system has also been suggested as a mechanism promoting discordant immune responses.[69,70] Indeed, untreated HIV infection leads to a significant activation of the immune system,[59,71] resulting in generalized and systemic inflammation, persistent T-cell activa-

may constitute relevant therapeutic alternatives as second- and third-line ART regimens for HIV-1-infected children and adolescents in therapeutic or virological failure living in sub-Saharan Africa.[95]

In conclusion, basing treatment decisions exclusively on immunological parameters would lead to unnecessary treatment switches in a substantial number of patients, as previously emphasized.[92–94] Thus, close biological monitoring with access to routine plasma HIV-1 RNA load and CD4 T-cell count monitoring is crucial, despite being difficult in resource-con-strained countries, and constitutes a strong necessity for adapting the complex outcomes of ART in HIV-1-infected children born from infected mothers.

Acknowledgments

Author contributions

Conceptualization: Christian Diamant Mossoro-Kpinde, Jean Chrysostome Gody, Ralph-Sydney Mboumba Bouassa, Laurent Bélec.
Data curation: Sandrine Moussa, Jean De Dieu Longo.
Formal analysis: Christian Diamant Mossoro-Kpinde, Ralph-Sydney Mboumba Bouassa, Sandrine Moussa, Mathieu Matta.
Investigation: Laurent Bélec.
Methodology: Ralph-Sydney Mboumba Bouassa.
Project administration: Jean Chrysostome Gody.
Supervision: Jean De Dieu Longo, Gérard Grésenguet, Joël Fleury Djoba Siawaya, Laurent Bélec.
Validation: Jean De Dieu Longo, Gérard Grésenguet, Joël Fleury Djoba Siawaya, Laurent Bélec.
Writing – original draft: Christian Diamant Mossoro-Kpinde, Ralph-Sydney Mboumba Bouassa, Mohammad-Ali Jenabian, Hélène Péré, Charlotte Charpentier, Laurent Bélec.
Writing – review & editing: Christian Diamant Mossoro-Kpinde, Ralph-Sydney Mboumba Bouassa, Hélène Péré, Charlotte Charpentier, Joël Fleury Djoba Siawaya, Laurent Bélec.

References

[1] UNAIDS Global HIV & AIDS Statistics — 2018 Fact Sheet. Available at: http://www.unaids.org/en/resources/fact-sheet (Accessed December 30, 2018)

[2] World Health Organization. Child Mortality (Updated September 2011). Available at: http://www.who.int/pmnch/media/press_materials/fs/fs_mdg4_childmortality/en/. (Accessed January 1, 2019)

[3] Newell ML, Coovadia H, Cortina-Borja M, et al. Ghent International AIDS Society (IAS) Working Group on HIV Infection in Women and Children.Mortality of infected and uninfected infants born to HIV-infected mothers in Africa: a pooled analysis. Lancet 2004;364:1236–43.

[4] UNAIDS. Ending AIDS: Progress Towards the 90-90-90 Targets; 2017. Available at: http://www.unaids.org/en/resources/documents/2017/20170720_Global_AIDS_update_2017. (Accessed January 05, 2019)

[5] Children & AIDS: 2015 Satistical Update (Updated November 2015). Available at: http://data.unicef.org/corecode/uploads/document6/uploaded_pdfs/corecode/2015-Children-Adolescents-and-AIDS-Statistical-Update-Executive-Summary_244.pdf. (Accessed January 1, 2019)

[6] van Rossum AM, Fraaij PL, de Groot R. Efficacy of highly active antiretroviral therapy in HIV-1 infected children. Lancet Infect Dis 2002;2:93–102.

[7] Scherpbier HJ, Bekker V, van Leth F, et al. Long-term experience with combination antiretroviral therapy that contains nelfinavir for up to 7 years in a pediatric cohort. Pediatrics 2006;117:E528–36.

[8] Resino S, Bellon JM, Ramos JT, et al. Impact of highly active antiretroviral therapy on CD4+ T cells and viral load of children with AIDS: a population- based study. AIDS Res Hum Retrovirus 2004; 20:927–31.

[9] Fraaij PL, Verweel G, van Rossum AM, et al. Sustained viral suppression and immune recovery in HIV type 1-infected children after 4 years of highly active antiretroviral therapy. Clin Infect Dis 2005;40:604–8.

[10] Salou M, Butel C, Konou AA, et al. High rates of drug resistance among newly diagnosed HIV-infected children in the national prevention of mother-to-child transmission program in Togo. Pediatr Infect Dis J Aug 2016;35:879–85.

[11] Jenabian MA, Costiniuk CT, Mboumba Bouassa RS, et al. Tackling virological failure in HIV-infected children living in Africa. Expert Rev Anti Infect Ther 2015;13:1213–23.

[12] Chaix ML, Rouet F, Kouakoussui KA, et al. Genotypic human immunodeficiency virus type 1 drug resistance in highly active antiretroviral therapy-treated children in Abidjan, Côte d'Ivoire. Pediatr Infect Dis J 2005;24:1072–6.

[13] Rouet F, Fassinou P, Inwoley A, et al. ANRS 1244/1278 Programme Enfants Yopougon. Long-term survival and immuno-virological re-sponse of African HIV-1-infected children to highly active antiretroviral therapy regimens. AIDS 2006;20:2315–9.

[14] Adjé-Touré C, Hanson DL, Talla-Nzussouo N, et al. Virologic and immunologic response to antiretroviral therapy and predictors of HIV type 1 drug resistance in children receiving treatment in Abidjan, Côte d'Ivoire. AIDS Res Hum Retroviruses 2008;24:911–7.

[15] Germanaud D, Derache A, Traore M, et al. Level of viral load and antiretroviral resistance after 6 months of non-nucleoside reverse transcriptase inhibitor first-line treatment in HIV-1-infected children in Mali. J Antimicrob Chemother 2010;65:118–24.

[16] Ahoua L, Guenther G, Rouzioux C, et al. Immunovirological response to combined antiretroviral therapy and drug resistance patterns in children: 1- and 2-year outcomes in rural Uganda. BMC Pediatr 2011;11:67. doi: 10.1186/1471-2431-11-67.

[17] Barth RE, Tempelman HA, Moraba R, et al. Long-term outcome of an HIV-treatment programme in rural Africa: viral suppression despite early mortality. AIDS Res Treat 2011;2011:434375. doi: 10.1155/2011/434375. Epub 2010 Nov 21.

[18] Barth RE, Tempelman HA, Smelt E, et al. Long-term outcome of children receiving antiretroviral treatment in rural South Africa: substantial virologic failure on first-line treatment. Pediatr Infect Dis J 2011;30: 52–6.

[19] Barlow-Mosha LN, Bagenda DS, Mudiope PK, et al. The long-term effectiveness of generic adult fixed-dose combination antiretroviral therapy for HIV-infected Ugandan children. Afr Health Sci 2012; 12:249–58.

[20] Barry O, Powell J, Renner L, et al. Effectiveness of first-line antiretroviral therapy and correlates of longitudinal changes in CD4 and viral load among HIV-infected children in Ghana. BMC Infect Dis Oct 13 2013;13:476. doi: 10.1186/1471-2334-13-476.

[21] Schoffelen AF, Wensing AM, Tempelman HA, et al. Sustained virological response on second-line antiretroviral therapy following virological failure in HIV-infected patients in rural South Africa. PLoS One 2013;8: e58526. doi: 10.1371/journal.pone.0058526. Epub 2013 Mar 11.

[22] Wamalwa DC, Lehman DA, Benki-Nugent S, et al. Long-term virologic response and genotypic resistance mutations in HIV-1 infected Kenyan children on combination antiretroviral therapy. J Acquir Immune Defic Syndr 2013;62:267–74.

[23] Salazar-Vizcaya L, Keiser O, Karl Technau, et al. Viral load versus CD4+ monitoring and 5-year outcomes of antiretroviral therapy in HIV-positive children in Southern Africa: a cohort-based modelling study. AIDS 2014;28:2451–60.

Mossoro-Kpinde et al. Medicine (2020) 99:21

Medicine

[24] Kukoyi O, Renner L, Powell J, et al. Viral load monitoring and antiretroviral treatment outcomes in a pediatric HIV cohort in Ghana. BMC Infect Dis 2016;16:58. doi: 10.1186/s12879-016-1402-9.

[25] Muri L, Gamell A, Ntamatungiro AJ, et al. Development of HIV drug resistance and therapeutic failure in children and adolescents in rural Tanzania: an emerging public health concern. AIDS 2017;31:61–70.

[26] Gody JC, Charpentier C, Mbitikon O, et al. High prevalence of antiretroviral drug resistance mutations in HIV-1 non-B subtype strains from African children receiving antiretroviral therapy regimen according to the 2006 revised WHO recommendations. J Acquir Immune Defic Syndr 2008;49:566–9.

[27] Charpentier C, Gody JC, Mbitikon O, et al. Virological response and resistance profiles after 18 to 30 months of first- or second-/third-line antiretroviral treatment: a cross-sectional evaluation in HIV type 1-infected children living in the Central African Republic. AIDS Res Hum Retroviruses 2012;28:87–94.

[28] Mossoro-Kpinde CD, Gody JC, Mboumba Bouassa RS, et al. High levels of virological failure with major genotypic resistance mutations in HIV-1-infected children after 5 years of care according to WHO-recommended 1st-line and 2nd-line antiretroviral regimens in the Central African Republic: a cross-sectional study. Medicine (Baltimore) 2017;96:e6282. doi: 10.1097/MD.0000000000006282.

[29] Green A. The Central African Republic's silent health crisis. Lancet 2012;380:964–5.

[30] Bélec L, Mbopi-Kéou FX. HIV epidemic out of control in Central African Republic. Lancet 2012;380:1993–4.

[31] World Health Organization (WHO, 2006) Recommendations. Antiretroviral Therapy of HIV Infection in Infants and Children in Resource-limited Settings, Towards Universal Access: Recommendations for a Public Health Approach. Geneva: WHO, 2006. Available at: http://www.who.int/hiv/pub/guidelines/WHOpaediatric.pdf. (Accessed August 30, 2017)

[32] World Health Organization (WHO, 2006) Recommendations. Antiretroviral Therapy of HIV Infection in Adults and Adolescents in Resource-limited Settings: Recommendations for a Public Health Approach (2006 Revision). Geneva: WHO, 2006. Available at: http://www.who.int/hiv/pub/guidelines/adult/en/. (Accessed August 30, 2017)

[33] World Health Organization (WHO, 2010) Recommendations. Antiretroviral Therapy for HIV Infection in Adults and Adolescents. Recommendations for a Public Health Approach: 2010 Revision. Geneva: WHO, 2010. Available at: https://www.who.int/hiv/pub/arv/adult2010/en/. (Accessed January 05, 2019)

[34] World Health Organization (WHO, 2013) Recommendations. Consolidated Guidelines on the Use of Antiretroviral Drugs for Treating and Preventing HIV Infection: Recommendations for a Public Health Approach. June 2013. Available at: http://www.who.int/hiv/pub/guidelines/arv2013/download/en/. (Accessed March 30, 2016)

[35] World Health Organization (WHO, 2014) Supplement. March 2014 Supplement to the 2013 Consolidated Guidelines on the Use of Antiretroviral Drugs for Treating and Preventing HIV Infection. Recommendations for a Public Health approach. Available at: https://www.who.int/hiv/pub/guidelines/arv2013/arvs2013upplement_march2014/en/. (Accessed January 1, 2019)

[36] World Health Organization (WHO, 2015). Consolidated Guidelines on the Use of Antiretroviral Drugs for Treating and Preventing HIV Infection: Recommendations for a Public Health Approach. Available at: http://www.who.int/hiv/pub/arv/policy-brief-arv-2015/en/. (Accessed August 30 2017)

[37] Mossoro-Kpinde CD, Mboumba Bouassa RS, Jenabian MA, et al. Analytical performances of human immunodeficiency virus type 1 RNA-based Amplix® real-time PCR platform for HIV-1 RNA quantification. AIDS Res Treat 2016;2016:7954810. doi: 10.1155/2016/7954810. Epub 2016 Dec 5.

[38] World Health Organization (WHO, 2016). Consolidated Guidelines on the Use of Antiretroviral Drugs for Treating and Preventing HIV Infection: Recommendations for a Public Health Approach. Second Edition 2016. Available at: http://apps.who.int/iris/bitstream/10665/208825/1/9789241549684_eng.pdf. (Accessed August 30, 2017)

[39] World Health Organization (WHO, 2018) Technical Report. HIV Diagnosis and ARV use in HIV-Exposed Infants: a Programmatic Update. July 2018. Available at: http://apps.who.int/iris/bitstream/handle/10665/273155/WHO-CDS-HIV-18.17-eng.pdf?ua=1. (Accessed January 05, 2019)

[40] Mbopi-Kéou FX, Mion S, Sagnia B, et al. Validation of a single-platform, volumetric, CD45-assisted panleucogating Auto40 flow cytometer to determine the absolute number and percentages of CD4 T cells in resource-constrained settings using cameroonian patients' samples. Clin Vaccine Immunol 2012;19:609–15.

[41] Mossoro-Kpinde CD, Kouabosso A, Mboumba Bouassa RS, et al. Performance evaluation of the touchscreen-based Muse™ Auto CD4/CD4% single-platform system for CD4 T cell numeration in absolute number and in percentage using blood samples from children and adult patients living in the Central African Republic. J Transl Med 2016;14:326. doi: 10.1186/s12967-016-1082-7.

[42] Natesampillai S, Nie Z, Cummins NW, et al. Patients with discordant responses to antiretroviral therapy have impaired killing of HIV-infected T cells. PLoS Pathog 2010;6:e1001213. doi: 10.1371/journal.ppat.1001213.

[43] Bélec L, Bonn JP. Challenges in implementing HIV laboratory monitoring in resource-constrained settings: how to do more with less. Future Microbiol 2011;6:1251–60.

[44] Fassinou P, Elenga N, Rouet F, et al. Highly active antiretroviral therapies among HIV-1-infected children in Abidjan, Côte d'Ivoire. AIDS 2004;18:1905–13.

[45] O'Brien DP, Sauvageot D, Olson D, et al. Treatment outcomes stratified by baseline immunological status among young children receiving nonnucleoside reverse-transcriptase inhibitor-based antiretroviral therapy in resource-limited settings. Clin Infect Dis 2007;44:1245–8.

[46] Wamalwa DC, Farquhar C, Obimbo EM, et al. Early response to highly active antiretroviral therapy in HIV-1-infected Kenyan children. J Acquir Immune Defic Syndr 2007;45:311–7.

[47] Davies MA, Keiser O, Technau K, et al. Outcomes of the South African national antiretroviral treatment programme for children: the IeDEA Southern Africa collaboration. S Afr Med J 2009;99:730–7.

[48] Puthanakit T, Kerr S, Ananworanich J, et al. Pattern and predictors of immunologic recovery in human immunodeficiency virus-infected children receiving non-nucleoside reverse transcriptase inhibitor-based highly active antiretroviral therapy. Pediatr Infect Dis J 2009;28:488–92.

[49] Mugyenyi P, Walker AS, Hakim J, et al. DART Trial TeamRoutine versus clinically driven laboratory monitoring of HIV antiretroviral therapy in Africa (DART): a randomised non-inferiority trial. Lancet 2010;375:123–31.

[50] Kamya MR, Mayanja-Kizza H, Kambugu A, et al. Academic Alliance for AIDS Care and Prevention in AfricaPredictors of long-term viral failure among Ugandan children and adults treated with antiretroviral therapy. J Acquir Immune Defic Syndr 2007;46:187–93.

[51] Reddi A, Leeper SC, Grobler AC, et al. Preliminary outcomes of a paediatric highly active antiretroviral therapy cohort from KwaZulu-Natal, South Africa. BMC Pediatr 2007;7:13. doi: 10.1186/1471-2431-7-13.

[52] Bratholm C, Johannessen A, Naman E, et al. Drug resistance is widespread among children who receive long-term antiretroviral treatment at a rural Tanzanian hospital. J Antimicrob Chemother 2010;65:1996–2000.

[53] Emmett SD, Cunningham CK, Mmbaga BT, et al. Predicting virologic failure among HIV-1-infected children receiving antiretroviral therapy in Tanzania: a cross-sectional study. J Acquir Immune Defic Syndr 2010;54:368–75.

[54] Sigaloff KC, Calis JC, Geelen SP, et al. HIV-1-resistance-associated mutations after failure of first-line antiretroviral treatment among children in resource-poor regions: a systematic review. Lancet Infect Dis 2011;11:769–79.

[55] Zoufaly A, Fillekes Q, Hammerl R, et al. Prevalence and determinants of virological failure in HIV-infected children on antiretroviral therapy in rural Cameroon: a cross-sectional study. Antivir Ther 2013;18:681–90.

[56] Salou M, Dagnra AY, Butel C, et al. High rates of virological failure and drug resistance in perinatally HIV-1-infected children and adolescents receiving lifelong antiretroviral therapy in routine clinics in Togo. J Int AIDS Soc 2016;19:20683. doi: 10.7448/IAS.19.1.20683. eCollection 2016.

[57] Kebe K, Thiam M, Diagne Gueye NR, et al. High rate of antiretroviral drug resistance mutations in HIV type 1-infected Senegalese children in virological failure on first-line treatment according to the World Health Organization Guidelines. AIDS Res Hum Retroviruses 2013;29:242–9.

[58] Bandera A, Ferrario G, Saresella M, et al. CD4+ T cell depletion, immune activation and increased production of regulatory T cells in the thymus of HIV-infected individuals. PLoS One 2010;5:e10788. doi: 10.1371/journal.pone.0010788.

[59] Cotugno N, Douagi I, Rossi P, et al. Suboptimal immune reconstitution in vertically HIV infected children: a view on how HIV replication and

timing of HAART initiation can impact on T and B-cell compartment. Clin Dev Immunol 2012;2012:805151. doi: 10.1155/2012/805151. Epub 2012 Apr 8.

[60] Kelly C, Gaskell KM, Richardson M, et al. Discordant immune response with antiretroviral therapy in HIV-1: a systematic review of clinical outcomes. PLoS One 2016;11:e0156099. doi: 10.1371/journal.pone. 0156099.

[61] Deeks SG, Hoh R, Grant RM, et al. CD4+ T cell kinetics and activation in human immunodeficiency virus-infected patients who remain viremic despite long-term treatment with protease inhibitor-based therapy. J Infect Dis 2002;185:315–23.

[62] Bélec L, Piketty C, Si-Mohamed A, et al. High levels of drug-resistant human immunodeficiency virus variants in patients exhibiting increasing CD4+ T cell counts despite virologic failure of protease inhibitor-containing antiretroviral combination therapy. J Infect Dis 2000; 181:1808–12.

[63] Fessel WJ, Krowka JF, Sheppard HW, et al. Dissociation of immunologic and virologic responses to highly active antiretroviral therapy. J Acquir Immune Defic Syndr 2000;23:314–20.

[64] Piketty C, Weiss L, Thomas F, et al. Long-term clinical outcome of human immunodeficiency virus-infected patients with discordant immunologic and virologic responses to a protease inhibitor-containing regimen. J Infect Dis 2001;183:1328–35.

[65] Tuboi SH, Pacheco AG, Harrison LH, et al. ARTLINC of IeDEAMortality associated with discordant responses to antiretroviral therapy in resource-constrained settings. J Acquir Immune Defic Syndr 2010;53:70–7.

[66] Ometto L, De Forni D, Patiri F, et al. Immune reconstitution in HIV-1-infected children on antiretroviral therapy: role of thymic output and viral fitness. AIDS 2002;16:839–49.

[67] Vrisekoop N, van Gent R, de Boer AB, et al. Restoration of the CD4 T cell compartment after long-term highly active antiretroviral therapy without phenotypical signs of accelerated immunological aging. J Immunol 2008;181:1573–81.

[68] Li T, Wu N, Dai Y, et al. Reduced thymic output is a major mechanism of immune reconstitution failure in HIV-infected patients after long-term antiretroviral therapy. Clin Infect Dis 2011;53:944–51.

[69] Gandhi RT, Spritzler J, Chan E, et al. ACTG 384 TeamEffect of baseline-and treatment-related factors on immunologic recovery after initiation of antiretroviral therapy in HIV-1-positive subjects: results from ACTG 384. J Acquir Immune Defic Syndr 2006;42:426–34.

[70] Hunt PW, Cao HL, Muzoora C, et al. Impact of CD8+ T-cell activation on CD4+ T-cell recovery and mortality in HIV-infected Ugandans initiating antiretroviral therapy. AIDS 2011;25:2123–31.

[71] Serrano-Villar S, Sainz T, Lee SA, et al. HIV-infected individuals with low CD4/CD8 ratio despite effective antiretroviral therapy exhibit altered T cell subsets, heightened CD8+ T cell activation, and increased risk of non-AIDS morbidity and mortality. PLoS Pathog 2014;10:e1004078.

[72] Delobel P, Nugeyre MT, Cazabat M, et al. Naive T-cell depletion related to infection by X4 human immunodeficiency virus type 1 in poor immunological responders to highly active antiretroviral therapy. J Virol 2006;80:10229–36.

[73] Funderburg NT, Andrade A, Chan ES, et al. Dynamics of immune reconstitution and activation markers in HIV+ treatment-naïve patients treated with raltegravir, tenofovir disoproxil fumarate and emtricitabine. PLoS One 2013;8:e83514. doi: 10.1371/journal.pone.0083514.

[74] Shive CL, Mudd JC, Funderburg NT, et al. Inflammatory cytokines drive CD4+ T-cell cycling and impaired responsiveness to interleukin 7: implications for immune failure in HIV disease. J Infect Dis 2014; 210:619–29.

[75] Lapadula G, Chatenoud L, Gori A, et al. Risk of severe non AIDS events is increased among patients unable to increase their CD4+ T-cell counts >200+/µl despite effective HAART. PLoS One 2015;10:e0124741. doi: 10.1371/journal.pone.0124741. eCollection 2015.

[76] Kuller LH, Tracy R, Belloso W, et al. Inflammatory and coagulation biomarkers and mortality in patients with HIV infection. PLoS Med 2008;5:e203. doi: 10.1371/journal.pmed.0050203.

[77] Lawn SD, Little F, Bekker LG, et al. Changing mortality risk associated with CD4 cell response to antiretroviral therapy in South Africa. AIDS 2009;23:335–42.

[78] Mocroft A, Furrer HJ, Miro JM, et al. Opportunistic Infections Working Group on behalf of the Collaboration of Observational HIV Epidemiological Research Europe (COHERE) study in EuroCOORDThe incidence of AIDS-defining illnesses at a current CD4 count ≥ 200 cells/µL in the post-combination antiretroviral therapy era. Clin Infect Dis 2013;57:1038–47.

[79] Hainaut M, Verscheure V, Ducarme M, et al. Cellular immune responses in human immunodeficiency virus (HIV)-1-infected children: is immune restoration by highly active anti-retroviral therapy comparable to non-progression? Clin Exp Immunol 2011;165:77–84.

[80] Sufka SA, Ferrari G, Gryszowka VE, et al. Prolonged CD4+ cell/virus load discordance during treatment with protease inhibitor-based highly active antiretroviral therapy: immune response and viral control. J Infect Dis 2003;187:1027–37.

[81] Douek DC, Koup RA, McFarland RD, et al. Effect of HIV on thymic function before and after antiretroviral therapy in children. J Infect Dis 2000;181:1479–82.

[82] Chavan S, Bennuri B, Kharbanda M, et al. Evaluation of T cell receptor gene rearrangement excision circles after antiretroviral therapy in children infected with human immunodeficiency virus. J Infect Dis 2001;183:1445–54.

[83] De Rossi A, Walker AS, Klein N, et al. Increased thymic output after initiation of antiretroviral therapy in human immunodeficiency virus type 1-infected children in the Paediatric European Network for Treatment of AIDS (PENTA) 5 Trial. J Infect Dis 2002;186:312–20.

[84] De Rossi A. Virological and immunological response to antiretroviral therapy in HIV-1 infected children: genotypic and phenotypic assays in monitoring virological failure. New Microbiol 2004;27(2 Suppl 1): 45–50.

[85] Deeks SG, Barbour JD, Grant RM, et al. Duration and predictors of CD4 T-cell gains in patients who continue combination therapy despite detectable plasma viremia. AIDS 2002;16:201–7.

[86] Deeks SG, Hecht FM, Swanson M, et al. HIV RNA and CD4 cell count response to protease inhibitor therapy in an urban AIDS clinic: response to both initial and salvage therapy. AIDS 1999;13:F35–43.

[87] Mammano F, Petit C, Clavel F. Resistance-associated loss of viral fitness in human immunodeficiency virus type 1: phenotypic analysis of protease and gag coevolution in protease inhibitor-treated patients. J Virol 1998;72:7632–7.

[88] Zennou V, Mammano F, Paulous S, et al. Loss of viral fitness associated with multiple Gag and Gag-Pol processing defects in human immunodeficiency virus type 1 variants selected for resistance to protease inhibitors in vivo. J Virol 1998;72:3300–6.

[89] Mammano F, Trouplin V, Zennou V, et al. Retracing the evolutionary pathways of human immunodeficiency virus type 1 resistance to protease inhibitors: virus fitness in the absence and in the presence of drug. J Virol 2000;74:8524–31.

[90] Prado JG, Prado JG, Parkin NT, et al. HIV type 1 fitness evolution in antiretroviral-experienced patients with sustained CD4+ T cell counts but persistent virologic failure. Clin Infect Dis 2005;41:729–37.

[91] Martinez-Picado J, Martínez MA. HIV-1 reverse transcriptase inhibitor resistance mutations and fitness: a view from the clinic and ex vivo. Virus Res 2008;134:104–23.

[92] Reynolds SJ, Nakigozi G, Newell K, et al. Failure of immunologic criteria to appropriately identify antiretroviral treatment failure in Uganda. Aids 2009;23:697–700.

[93] Kantor R, Diero L, Delong A, et al. Misclassification of first-line antiretroviral treatment failure based on immunological monitoring of HIV infection in resource-limited settings. Clin Infect Dis 2009;49: 454–62.

[94] van Oosterhout JJ, Brown L, Weigel R, et al. Diagnosis of antiretroviral therapy failure in Malawi: poor performance of clinical and immunological WHO criteria. Trop Med Int Health 2009;14: 856–61.

[95] Mboumba Bouassa RS, Mossoro-Kpinde CD, Gody JC, et al. 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. J Antimicrob Chemother 2019;74:2030–8.