



Immunovirological long-term
outcome of HIV-infected children
living in a resource-limited setting
of South Africa:
Implication of an early treatment
initiation



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Abbreviations

AIDS	Acquired ImmunoDeficiency Syndrome
Anti-HBc	Anti-HBc antibodies
Anti-HBs	Anti-HBs antibodies
ARV	Antiretroviral
cART	Combination AntiRetroviral Therapy
CD4 ⁺ %	Percentage of total lymphocytes that are CD4 cells
DNA	DeoxyriboNucleic Acid
EPI	Expanded Program of Immunization
ESC	Early Starters Cohort
FDA	Food and Drug Administration
FDC	Fixed-Dose Combination
HBsAg	HBs antigen
HBV	Hepatitis B Virus
HIS	High Immunologically responding Subgroup
HIV	Human Immunodeficiency Virus
HIVDR	HIV drug resistance
IIS	Intermediate Immunologically responding Subgroup
INI	Integrase Inhibitor
KZN	KwaZulu-Natal
LIS	Low Immunologically responding Subgroup

LSC	Late Starters Cohort
MTCT	Mother-To-Child Transmission
NRTI	Nucleoside Reverse Transcriptase Inhibitor
NNRTI	Non-Nucleoside Reverse Transcriptase Inhibitor
OI	Opportunistic infection
PCR	Polymerase Chain Reaction
PDR	PreDrug Resistance
PENTA	Paediatric European Network for Treatment of AIDS
PI	Protease Inhibitor
PMTCT	Prevention of the Mother to Child Transmission
RLS	Resource-Limited Setting
RNA	RiboNucleic Acid
SA	South Africa
SSA	Sub-Saharan Africa
TB	Tuberculosis
UNAIDS	United Nations Programme on HIV/AIDS
US	United States National Institutes of Health
VF	Virologic Failure
VL	Viral Load
WHO	World Health Organization

I. Introduction

I. Are all HIV-infected children living in the same world?

The unequal distribution of HIV

In 2017, 36.9 million people were living with human immunodeficiency virus (HIV) infection¹. Sub-Saharan Africa (SSA) remained the most severely affected continent, with nearly 1 in every 25 adults (4.2%) living with HIV and accounting for nearly two-thirds of the people living with HIV worldwide² (Fig 1). With 7.2 million HIV infected people, South Africa (SA) has the largest HIV epidemic in the world³.

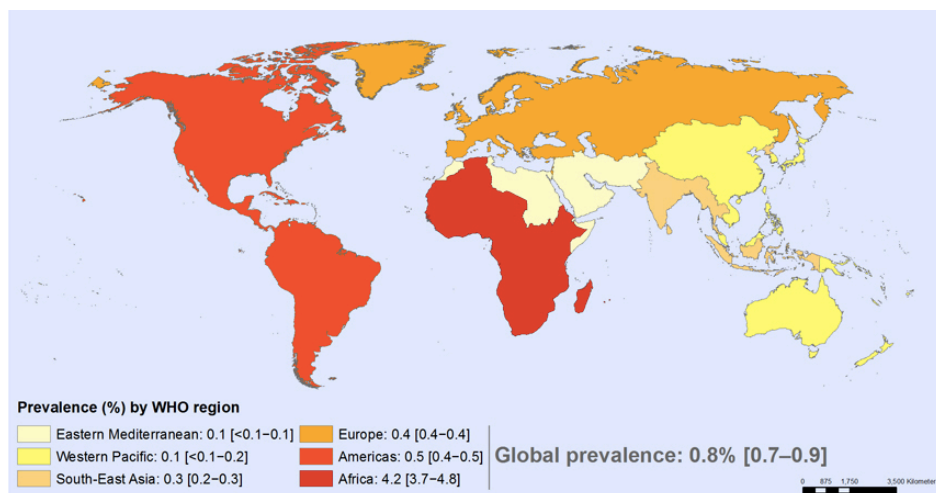


Figure 1. Prevalence of HIV among adults 15 to 49 by WHO region, 2017

Among these 36.9 million HIV infected people, approximately 2.1 million (5.7%) were children and 1.9 million (90%) of them were living in Africa⁴.

In SA, 320.000 (260.000 – 400.000) children were living with HIV in 2016 and 12.000 (9.600 – 22.000) of them were newly infected due to mother-to-child transmission (MTCT)³.

Towards a greater access to antiretroviral therapy

Between 2000 and 2016, children receiving lifelong combination antiretroviral therapy (cART) worldwide increased from 14.900 to 872.500, which represents 43% of the HIV-infected pediatric population⁵. In comparison, 54% of the HIV-infected adults received lifelong cART in 2016⁶.

However, great disparities persist between resource-rich settings like western countries where more than 95% of children have access to cART and resource-limited settings (RLS) like Africa or the Middle East where only 20% of children have access to cART⁷.

In SA, 172.000 children were receiving cART in 2016 and 25.000 newly infected children initiated a treatment, which represented a treatment coverage of 55% (45 – 70%)³.

Is treatment adapted for children?

Currently, 25 HIV ARV drugs and 2 PK enhancers have received approval by the Food and Drug Administration (FDA) for use in HIV-infected adults and adolescents⁸ (Table1). Remarkably, 23 of them are presently approved by the FDA for pediatric use⁸ compared to 17 in 2009⁹. But only 15 of these 23 antiretroviral (ARV) drugs have adapted formulations (oral solutions, oral powder, chewable tablets, or injections) for children.

In SA, five Nucleoside/Nucleotide Reverse Transcriptase Inhibitors (NRTIs/NtRTIs) (ABC, 3TC, AZT, FTC and TDF), one Protease Inhibitor (PI) (LPV/r) and two Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTI) (NVP and EFV) are presently used for the treatment of children. Two more NRTI are available but are no more recommended (d4T, and ddI)¹⁰.

Class	Drug	Abbreviation	FDA approval
NRTIs/NtRTIs	Abacavir	ABC	≥ 3 months
	Didanosine	ddI	≥ 2 weeks
	Emtricitabine	FTC	From birth
	Lamivudine	3TC	From birth
	Stavudine	d4T	From birth
	Tenofovir disoproxil fumarate	TDF	≥ 2 years
	Tenofovir alafenamide fumarate	TAF	≥ 12 years + ≥ 35 kg
	Zidovudine	AZT	From birth
NNRTIs	Efavirenz	EFV	≥ 3 months + ≥ 3 kg
	Etravirine	ETR	≥ 6 years
	Nevirapine	NVP	≥ 1 months*
	Rilpivirine	RPV	≥ 12 years + ≥ 35 kg
PIs	Atazanavir	ATV	≥ 3 months + ≥ 5 kg
	Darunavir	DRV	≥ 3 years + ≥ 10 kg
	Fosamprenavir	FPV	≥ 6 months
	Indinavir	IDV	Not for children
	Lopinavir	LPV	≥ 2 weeks
	Nelfinavir	NFV	≥ 2 years
	Saquinavir	SQV	Not for children
	Tipranavir	TPV	≥ 2 years
Fusion inhibitors	Enfuvirtide	T20	≥ 6 years
	Maraviroc	MVC	≥ 2 years + ≥ 10 kg
INIs	Dolutegravir	DTG	≥ 30 kg
	Elvitegravir	EVG	≥ 35 kg
	Raltegravir	RAL	From birth

Table 1. HIV antiretroviral drugs currently approved by the FDA⁸.

* Investigational doses for neonates < 1 month of age exist but are not approved by the FDA.

NRTIs/NtRTIs: Nucleoside/Nucleotide Reverse Transcriptase Inhibitors, NNRTIs: Non-Nucleoside Reverse Transcriptase Inhibitors, PIs: Protease Inhibitors, INIs: Integrase Inhibitors

II. Early treatment initiation for HIV-infected infants and children

Benefits of early treatment initiation

The clinical symptoms and biological expression of HIV in children follow a bimodal distribution. In the absence of any ARV treatment, up to 52% of children born with HIV in RLS will die before the age of 2 years and 62% before the age of 5 years¹¹⁻¹⁴. Studies implemented in Europe 30 years ago, showed the same bimodal evolution but with a lower mortality rate (10% before 1 year and 28% before 5 years of age)¹⁵⁻¹⁶. By 5 years of age, the risk of mortality and disease progression in the absence of treatment falls to rates similar to those of young adults¹⁷.

The Children with HIV Early Antiretroviral Therapy (CHER) study compared the outcome of HIV-infected infants (6–12 weeks of age) who would have received deferred ART on the basis of clinical or immunological criteria with infants who immediately initiated cART after diagnosis. Immediate ART reduced early infant mortality by 76% and HIV progression by 75%¹⁸. Based on those data, the World Health Organization (WHO) recommended in 2010 an immediate cART initiation for children younger than 2 years¹⁹.

During the following years, the WHO recommended to start cART in all children younger than 5 years²⁰ and finally in all children independently of their age, clinical, immunological, or virological status²¹. This latest recommendation is based on the Strategic Timing of Antiretroviral Therapy (START) and the Trial of Early Antiretrovirals and Isoniazid Preventive Therapy in Africa (TEMPRANO) studies^{22,23}, which demonstrated benefits of cART initiation in asymptomatic adults regardless of CD4 cell count.

Early cART initiation allows control of viral replication before HIV evolved into more quasispecies, slows immune system destruction, preserves immune function²⁴ and limits the size of HIV-1 reservoirs^{25,26}. Moreover, early cART initiation induces fewer drug resistance mutations at virologic failure (VF) in adults due to a lower viral load (VL) at baseline, as detailed in the study HTPN 052^{27,28}. To our knowledge, this has not been demonstrated in children.

Between 2015 and 2017, European and American guidelines also recommended immediate cART initiation in all HIV-infected children before immunosuppression and occurrence of symptoms^{8,29,30}. Progressively, in people (adults and children) diagnosed with HIV, the overall median CD4 cell count in the world at enrolment in HIV care rose from 133 cells/ μ l in 2006 to 272 cells/ μ l in 2014 (Fig 2)³¹.

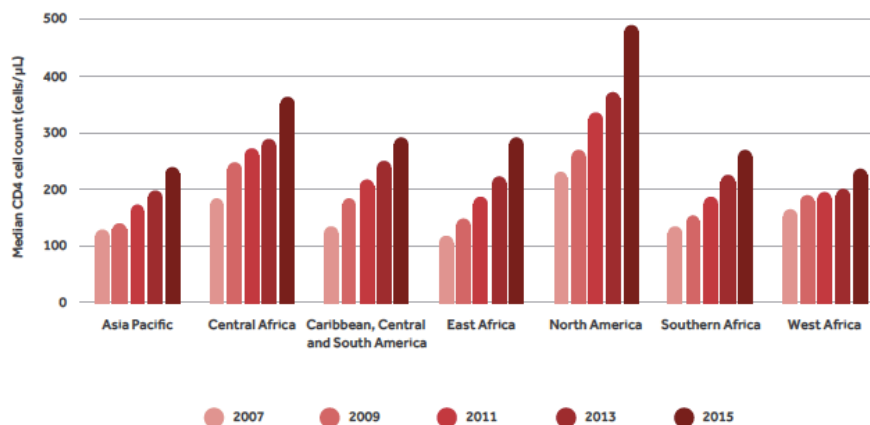


Figure 2 Median CD4 cell count at cART initiation in different parts of the world³¹

First line therapies in infants and children

According to the Paediatric European Network for Treatment of AIDS (PENTA), United States National Institutes of Health (US NIH) and WHO guidelines^{8,21,29}, the preferred treatment for HIV-infected children is a combination with at least three drugs including either a PI, NNRTI, or integrase inhibitor (INI) plus a dual NRTI backbone (Table 2).

	WHO	USA	Europe
≥12 years	TDF + 3TC/FTC + EFV	2 NRTIs ^a + ATV/r or DTG or DRV/r or EVG/cobicistat	TDF + FTC + ATV/r or DRV/r or EFV
6 - 12 years	ABC + 3TC + EFV	2NRTIs ^a + ATV/r or DTG	ABC + 3TC + EFV or ATV/r
3 - 6 years	ABC + 3TC + EFV	2NRTIs ^a + ATV/r or DRV/r or RAL	ABC + 3TC + EFV or LPV/r
≤ 3 years	ABC/AZT + 3TC + LPV/r	2NRTIs ^a + LPV/r or RAL or NVP ^b	ABC + 3TC + LPV/r or ABC + AZT + 3TC + NVP ^c

Table 2. First-line regimens recommended by the WHO, US NIH and PENTA guidelines^{8,21,29}. ABC : Abacavir, 3TC : Lamivudine, FTC : Emtricitabine, EFV : Efavirenz, AZT : Zidovudine, LPV/r : Ritonavir boosted Lopinavir, ATV/r : Ritonavir boosted Atazanavir, DTG : Dolutegravir, DRV : Darunavir, EVG : Elvitegravir, RAL : Raltegravir, NVP : Nevirapine, TDF : Tenofovir
^a< 12 years : ABC or AZT + 3TC or FTC; ≥ 12 years: ABC or TAF + 3TC or FTC (TAF always associated with FTC). ^bIf < 2 weeks old. ^cIn children < 3 years, add AZT to NVP-based regimens if high VL or central nervous system involvement until VL has been suppressed for at least 3 months. Four-drug induction for infants on NVP-based therapy may be considered until VL has been suppressed for at least 3 months, followed by three-drug maintenance therapy.

Despite the lack of direct comparison in children, the recommended NRTI backbones are ABC + 3TC or FTC between 3 months and 12 years of age. ABC + 3TC or TDF/TAF + FTC are recommended after 12 years of age^{8,21}. US NIH guidelines recommend AZT + 3TC before 3 months of age. FTC may replace 3TC.

In children younger than 3 years, a LPV/r based regimen is preferred to a NVP based regimen from 2 weeks of life on. It confers reduced risk of discontinuing treatment due to VF induced by NNRTI resistance^{32,33}, and decreases mortality regardless of previous NNRTI exposure^{34,35}. There are currently no data suggesting that initiating treatment within the first 2 weeks of life improves children's outcome⁸, but the US NIH recommends starting cART with

AZT + 3TC + NVP or AZT + 3TC + RAL if initiated before that age. There is no evidence that neonatal prophylaxis regimens optimized following maternal drug resistance are more effective than standard neonatal prophylaxis regimens⁸.

In children older than 3 years, the US NIH recently recommended the use of PIs or INIs as initial therapy, contrary to the WHO and PENTA guidelines which still recommend the use of PIs or NNRTIs^{8,21,29}. In RLS, EFV remains the only widely accessible drug for children older than 3 years, and it ensures harmonization of regimens across age groups²¹.

Current South African antiretroviral treatment guidelines recommend for all infants and children under 3 years of age (or <10 kg) to be treated with ABC, 3TC and LPV/r when they initiate their treatment. Children over 3 years of age are treated with ABC, 3TC and EFV¹⁰. Before 2010, guidelines recommended to start treatment with d4T instead of ABC³⁶.

Risk factors of treatment failure in infants

Infants depend entirely on their caregivers for ART administration. Most children with chronic illness are treated by their parents, which is often not the case with HIV because either mother and/or father may have died due to HIV. The characteristics of the new caregivers are associated with therapeutic success or failure. The impact of the caregiver's biologic relationship to the child was associated with better adherence in some studies^{37,38} and worse adherence in other³⁹. Caregivers who are also on ART may draw from their own experiences to support their child's adherence⁴⁰ and non-adherent HIV infected caregivers have more non-adherent children⁴¹. Presence of multiple caregivers was associated with poor adherence⁴². Finally, caregiver's age between 25 and 44 was associated with better adherence⁴³.

Transmitted and acquired viral resistances are other risk factors of treatment failure associated with early age at treatment initiation. Transmission of resistant viruses from the mother to child may occur in infancy during pregnancy, delivery and breastfeeding⁴⁴. Moreover, South African children born from HIV infected mothers have normally benefited from a prevention of the mother to child transmission (PMTCT) with NVP during 6 to 12 weeks. In case of failure of this prevention, infants are at high risk to acquire NNRTI resistances^{32,45,46}. Persistence of this resistance during the next years could lead to treatment failure in case of NNRTI based treatment. Finally, low-level drug concentration in children's blood due to their constant weight gain and socio-economical restrictions may also lead to treatment failures⁴⁷.

III. From a life threatening to a chronic disease

Due to the increased access to cART and early treatment initiation, pediatric mortality dramatically decreased from 570.000 in 2005 to 120.000 in 2016^{1,3}.

Early treatment initiation allowed more and more infants to reach childhood and adolescence. However, all of them have to face the difficulties linked with a daily and lifelong cART sometimes complicated by co-infection treatments.

Children, as in infancy, are facing limited choice of formulations, with poor palatability, large pill size or high pill burden, and unavailability of once-daily regimens^{48,49}. Dependence on adults may rapidly decrease with age and some young children take control of their treatment on early life.

Adherence in adolescents is influenced as in adults by common factors as forgetting doses, coping with complex medications, distance to health services,

long waiting times for prescription refills, and costs of care^{50,51}. However, other challenges including need to look like their peers, assuming increased responsibility for their own care, difficulties in navigating the health-care system and lack of links between adult and pediatric services are more specific to adolescents⁵²⁻⁵⁶. It is estimated that over one-third (38%) of adolescents globally are suboptimally adherent to ART, with substantial regional variation (16 to 60%)⁵⁴. Two Canadian^{57,58} studies focused on young adults infected during the perinatal period, during their transition from an adolescent to an adult HIV-clinic. The first found that 70% of transferring adolescent to adult clinics were failing treatment defined by detectable VL, CD4 counts < 200 and/or triple class drug resistance. The second reported 50 % of difficulty with adherence to the current drug regimens, a decrease in the proportion of patients with CD4 count >500 cells/mm from 64 to 29 % and a statistically significant decrease in absolute CD4 count (mean 370 vs 524 cells/mm) one year after transition. The majority (92 %) of participants felt that 18 years of age was too young to transfer to adult care. Few data on transition outcomes for HIV-infected adolescents are available from Africa settings. A review including 9 African studies (Southern and East Africa) was published in 2017. It described a lack of adequate infrastructure, staff training and communication between pediatric and adult clinicians as well as the fear of stigma of adolescents and youth living with HIV. Very high attrition was observed among adolescents and youth compared to younger children or older adults. Most countries had no specific national guidelines highlighting when and how to initiate transition to adult care. Transition tended to occur across a wide age range (13–22 years), with most occurring after 18 years⁵⁹. These studies highlight the importance of adapted care to prepare the transition from adolescent to adult HIV-clinics.

Finally, side effects of ARVs may limit their use at any age and are exposed in the next paragraphs.

LPV/r may induce gastro-intestinal symptoms (diarrhea, nausea and vomiting) headache, asthenia, rash, insulin resistance and hyperlipidemia (hypertriglyceridemia). Fat maldistribution is less common. LPV/r should not be used during the immediate postnatal period in premature infants because some case of adrenal insufficiency, bradyarrhythmias, cardiac dysfunction, lactic acidosis, acute renal failure, central nervous system and respiratory depression have been described. There are controversial data about less-robust weight gain and smaller increases in CD4 percentage for children treated with LPV/r in comparison with children treated with EFV⁸. Hyperlipidaemia is common in HIV-infected adults initiating LPV/r in RLS⁶⁰. Baseline lipid measurements and routine monitoring should be recommended in individuals starting LPV/r-based treatments with borderline high lipids⁶⁰.

EFV most common side effects are skin rash and increased transaminase levels. Rash was reported in up to 40% of children and 27% of adults. Onset is typically during the first 2 weeks of treatment. Although severe rash and Stevens-Johnson syndrome have been reported, they are rare. Many neurological symptoms (dizziness, somnolence, insomnia, abnormal dreams, hallucinations, seizures...) have also been reported. Rare cases of QTc prolongation have been observed⁸. There are increasing concerns about neurological side effects. Young HIV-exposed uninfected children may be at increased risk for cognitive and motor delay despite maternal ART and breastfeeding. Infants born preterm may be particularly vulnerable⁶¹. Another study showed that switching from EFV to RPV resulted in objective cognitive improvement on group level in cognitively asymptomatic patients⁶².

ABC may induce nausea, vomiting, fever, headache, diarrhea, rash, and anorexia. Hypersensitivity reactions have been observed in approximately 5% of adults and children (rate varies by race/ethnicity). Patients with HLA-B*5701 allele are at risk to develop this reaction and should not receive ABC. There are concerns about increased risk of myocardial infarction (in observational studies in adults)⁸.

TDF may cause nausea, diarrhea, vomiting, and flatulence. Bone toxicity (osteomalacia and reduced bone mineral density) was observed in both adults and children taking TDF. Renal toxicity, including increased serum creatinine, glycosuria, proteinuria, phosphaturia, and/or calciuria and decreased serum phosphate, has been observed⁸.

3TC rarely induce headache and nausea. Some cases of peripheral neuropathy, lipodystrophy/lipoatrophy and lactic acidosis have been described⁸.

Sustain optimal treatment and adherence remains a challenge for HIV infected children, their caregivers and the medical staff.

IV. Long-term benefits of an early treatment initiation

Immunological point of view

HIV infection causes a progressive depletion of CD4⁺ T-cell populations with progressive impairment of cellular immunity and increasing susceptibility to opportunistic infections (OIs)^{63,64}. In most adults, memory T-cell regeneration transiently preserves CD4⁺ T-cell numbers and functions above the threshold associated with immunodeficiency. Finally, continuous viral replication disturbs memory T-cell homeostasis and critical effector populations decline below the

level necessary to prevent OIs⁶⁴.

During early pediatric age, the total lymphocyte counts and the absolute CD4 T lymphocytes counts are higher than seen in the adult population. There is a gradual decrease in counts till they reach the adult normal range. Younger children normally have higher CD4 counts than older children and adults. CD4+ lymphocyte percentage (CD4%) is the preferred measurement in children <5 years old, because it varies less than in older children. At ≥ 5 years of age, either CD4% or absolute CD4 count can be used but CD4 count is preferred⁶⁵.

Some children (25-30%)¹¹ have a very early and severe disease (rapid progressors) and will die before the age of one year and others a slowly progressive one comparable to most of the adolescent and adults. Even infants with a high CD4⁺% at diagnosis are at higher risk of rapid immunosuppression than adults.

Many risk factors of rapid progression were identified. Maternal factors as low-level education, immunodeficiency and high VL during pregnancy and delivery were pointed out. For infants, lack of co-trimoxazole prophylaxis and absence of antiretroviral prophylaxis after birth were highlighted⁶⁶⁻⁶⁹.

Benefits of early treatment initiation on immunologic restoration, have been demonstrated in short term follow-up studies implemented in RLS and a review including 5 African studies, showed a 12 months CD4⁺% increase of 13.7%, for children initiating cART in RLS⁷⁰⁻⁷³.

Only a few studies focused on the long-term (>3 years) immunological outcome of HIV-infected children living in SSA⁷⁴⁻⁷⁸ despite a higher risk to develop treatment failure due to inappropriate antiretroviral formulations, adherence difficulties, drug toxicity or development of resistance³⁴.

Virological point of view in terms of viral load

In 2013, the WHO defined viral load (VL) testing as a better approach than clinical or immunological monitoring to diagnose and confirm treatment failure. VL provides a more accurate indication for the need to switch from first to second-line therapy, reduces the accumulation of drug resistance mutations and improves clinical outcomes^{1,79-81}. Although the vast majority of low and middle-income countries recommend routine VL testing, overall availability remains very low⁸². One estimate found that only 23% of the need for routine VL testing was met in 2013 with availability expected to increase to 47% by 2019⁸³.

In 2016, there were over 3900 National Department of Health clinics providing ART initiation and monitoring services in SA⁸⁴. Approximately 4 million VL were performed in these clinics and managed by only 16 centralized VL laboratories across the 9 provinces⁸⁵.

The latest 2017 United Nations Programme on HIV/AIDS (UNAIDS) report estimated that 86% of HIV-infected South African knew their HIV status but only 56% of them were on treatment. Globally, 45% of HIV infected people were virally suppressed but these proportion increased to 81% for people on treatment. For comparison, 84% of HIV-infected Belgian people knew their HIV status and 84% of them were on treatment with > 89% virally suppressed⁸⁶.

Between 2010 and 2016, the percentage of South African children living with HIV on treatment dramatically increased (29% to 55%) but only a few studies focused on the virological outcome of these children during that period⁸⁷⁻⁹¹.

Long-term resistances

Despite the high effectiveness of cART to control infection in HIV infected people, children stay at higher risk of VF^{92,93}.

VF during first line cART occurs in 11% to 19%^{88,94} of South African children (follow-up period : 8 to 11 years). Before changing to a second line cART, national guidelines¹⁰ recommend to assess and improve adherence. Many interventions were proposed in the literature as peer counselors, mobile phone text messages, reminder devices (alarms, calendar, etc.), cognitive behavioral therapy and behavioral skill training^{21,55}. Unfortunately these interventions are not always accessible in RLS and their effectiveness may be variable according to place⁵⁶.

South African and international guidelines highlighted the importance of monitoring HIV drug resistances (HIVDR). Indeed, HIVDR may put current cART regimens at risk and drive up the costs of successful treatments^{8,10,29,56}. Implanting HIV-1 genotypic resistance testing below the level of provincial or national laboratories remains a challenge in most of RLS⁹⁵. In case of persistent VF despite a reported good adherence, only children treated with a history of unboosted protease inhibitor regimen, tuberculosis treatment concomitant with boosted lopinavir regimen or second line cART failure may benefit from a resistance testing in the South African public system of health¹⁰.

A few studies used genotyping to determine the level of HIVDR in South African children^{89-91,93,96-98}. Only one of these⁹¹ was conducted in a rural setting and was limited to children with detectable VL, after failing first-line antiretroviral treatment.

Resistance testing after VF is recommended to guide clinicians to choose second or third lines of cART. The search for mutations in RNA is the gold standard.

HIV DNA needs to be integrated in human genome to be replicated and infect other human cells. Patients receiving effective cART, should have undetectable VL because they have no circulating virus. Even without circulating virus, viral genome may be detected in human cells (leucocytes for example) and studied after amplification. Testing proviral HIV-1 DNA before VF could present different advantages. It may be useful at the collective level to guide national recommendations for first and second line therapies⁹⁹. At the individual level, patients without VF but severe side effects may also benefit from proviral DNA detection before to switch to another therapy. Proviral DNA genotyping may guide the clinician when switching therapy due to VF⁹⁹, but may not be representative of the circulating virus (provirus may present more mutations than the circulating virus). Viral RNA genotyping stays the first choice to highlight viral mutations in case of detectable VL. In SA, children benefit from a national program of treatment without baseline resistance testing at cART initiation¹⁰ and resistance mutations to one or multiple drugs may already be present at baseline. Children with VF may receive ineffective new cART due to the presence of HIVDR in their provirus.

V. Children co-infected with HIV and other micro-organisms

The immunodeficiency caused by chronic HIV infection increases the risk of co-infection with pathogens that are normally controlled by innate and adaptive immune responses¹⁰⁰.

In SSA, rates of co-infections with bacteria (*M. tuberculosis*) viruses (hepatitis B virus (HBV), hepatitis C virus (HCV)), cytomegalovirus (CMV), yeasts (*C. neoformans*) or parasites (*P. falciparum*) remain particularly high¹⁰⁰.

Tuberculosis (TB) is the most common OI in individuals with HIV infection. There are many negative interactions between the two pathogens. The risk of developing tuberculosis is 16 to 27 times greater in people living with HIV than among those without HIV infection^{100,101}. TB accounts for 26% of acquired immunodeficiency syndrome (AIDS)-related deaths¹⁰⁰. Moreover, Rifampicine decreases the blood concentrations of LPV/r, which could lead to a cascade of treatment failure, immunosuppression and finally co-infection with OIs⁴⁷. Unfortunately, detection of a previous contact with TB by tuberculin testing is difficult due to BCG administration at birth in SA and most of children are treated on a clinical basis due to difficulties to grow these bacteria and lack of access to other laboratory tests like interferon gamma release assay (GIRA)¹⁰².

In contrast to *M. tuberculosis*, HBV contact or vaccination may be determined separately by serology. HBV affects 2–4 million (5-10%) of HIV infected people in the world. HIV/HBV co-infection worsens the natural progress of HBV with a significantly higher liver-related mortality compared to patients infected with HIV or HBV alone^{2,103-105}.

The prevalence of HBV remains high in the South African population (>8%) despite the implementation of an expanded program of vaccination against HBV in 1995. The latest available data (2012) about prevalence of chronic HBV infection in South African children, mention a high rate of chronic HBV carriage (5-7%)¹⁰⁶. Due to the absence of a national HBV serosurvey, data on the long-term impact of the vaccine on HIV-infected children are scarce.

VI. Objectives

The major objective of this thesis is to answer to the question: “On the long term, is early treatment initiation at least as effective as late treatment initiation in terms of CD4⁺%, viral load and viral resistances for children living in a RLS of SA?”

To answer this question, we will compare two cohorts of HIV infected children living in a RLS of SA. The first initiated cART before one year (Early Starters Cohort) and the second after two years of age (Late Starters Cohort). Immunological outcome (CD4⁺%), which is linked with the risk to develop OIs, will be studied in both cohorts and also in subgroups differing in CD4⁺% at treatment initiation. Virological outcomes (VL and resistances) will be compared in both cohorts and reflect the short and long-term effectiveness of cART.

Knowing the major short-term benefits of an early in life treatment initiation, recommendation to initiate cART as fast as possible after diagnosis could not be modified. However, data about long-term outcome of HIV-infected children who initiated their treatment before one year of age in RLS are capital. Unfavorable results could lead to a revision of the cART regimens and an improvement of the adherence strategies.

The second objective of this thesis is to answer to the question: “What is the prevalence of HIV/HBV coinfection and what is the effectiveness of the national program of vaccination for children living in a RLS of SA?”

As explained before, HBV prevalence is high in SA. HIV-infected children are normally vaccinated against HBV in infancy. There is however no screening for HBV infection or vaccination for children treated in HIV clinics. HBV serologies of two cohorts of HIV-infected and uninfected children will be compared.

II. Long-term
immunological and
virological outcomes :
is early treatment initiation
an advantage?

§1. Methodology

I. Study setting

This study was implemented between September and December 2014 in the province of Kwazulu-Natal (KZN), which had the highest HIV prevalence of SA in the latest national survey implemented in 2012¹⁰⁷ (Fig 3). The prevalence (39.3%) of HIV in pregnant women attending prenatal clinics was also the highest of SA in 2015¹⁰⁸.

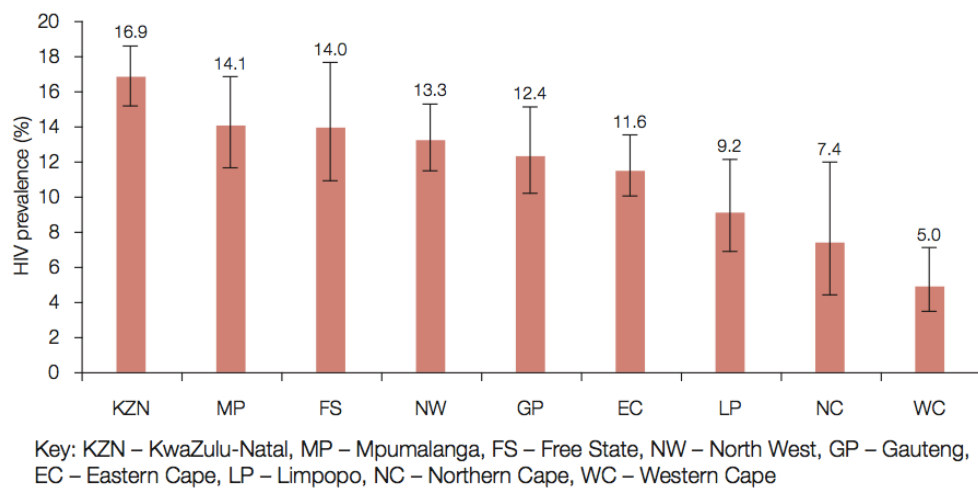


Figure 3. Overall HIV prevalence by province, South Africa 2012¹

Children were treated at the Edendale Regional Hospital located in a peri-urban area of Pietermaritzburg near Durban. A HIV family clinic was built in the compound of the hospital and provided care to approximately 3000 HIV-positive children and their mothers in 2014. Children and their families were living in townships near the hospital, an urban settlement for black occupation as defined by the apartheid legislation. There is a lot of poverty and many houses located in these townships have no access to running water or electricity.

II. Study population

Children in the Early Starters Cohort (ESC) were included in a previous study about feasibility and effectiveness of early cART initiation in RLS, which took place between 2005 and 2008¹⁰⁹. First condition to be included in this previous study was to be younger than 1 year of age at cART initiation (median 8.6 months, range 2.1–11.9 months). The other conditions to initiate cART included positive HIV-1 DNA Polymerase Chain Reaction (PCR), WHO stage 2-4 and/or CD4⁺% <30%. Children were included in the present study during a follow-up consultation at the HIV clinic.

Children included in the Late Starters Cohort (LSC) were invited to participate to the present study during a follow-up consultation at the HIV clinic. LSC children initiated cART after 2 years of age and had to be vertically infected. They were paired with ESC children according to their birth year or date of cART initiation (same year). The caregivers verbally confirmed the positive HIV status of the mothers during the inclusion in the present study. There were no laboratory results available to confirm it. Age at cART initiation had to be mentioned in the patient's file. These children were not included in the previous study between 2005 and 2008 because they were older than 1 year during this period or because they started cART after 2008. They initiated cART for free, according to their WHO clinical stage, CD4⁺% or comorbidities following the successive South African guidelines between 2005 and 2012.

Children from both cohorts were followed-up every month to every three months by the local staff between 2008 and 2014 and were treated according to the national recommendations with their antiretroviral drugs provided free of charge. Patients weight and height were measured at every consultation with clothes and many times values were just retyped from a consultation to another.

For these reasons, anthropometric parameters have not been taken into account in the present study. The staff was composed of two permanent pediatricians and two or three medical students, who were regularly changing.

III. Study procedures

Parents or caregivers gave a written informed consent prior to any procedure. A questionnaire was filled with the investigator and a nurse if translation was needed and blood samples were taken.

VL and CD4⁺% values(main outcome measures) were collected from the medical files (before 2010 records on paper and later on computer) because they were tested during follow-up consultation at least once a year. Different doctors completed these files during the years and some data were missing.

HIVDR (third outcome measure) were tested at the Université catholique de Louvain AIDS reference laboratory in Belgium because they are not tested in routine in SA. We tested proviral HIVDR in all patients to have comparable results between patients with detectable and undetectable VL.

The studies were approved by the South African ethical committee «Umgungundlovu Health District Review Board (UHERB)» and the Belgian Ethical committee «Comité d’Ethique Hospitalo-Facultaire Saint Luc – UCL».

All the results of these studies (serologies and genotyping) were transmitted to the local staff.

IV. Laboratory procedures

VL was measured at the Edendale Hospital laboratory by real time PCR, with the "COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 (Roche Diagnostics)" following the manufacturer's instructions. The lower limit of quantification (LLOQ) was 20 copies/ml.

One ml of whole blood was stored at -20°C in a private laboratory (Global Lab Durban) during a maximum of 3 months. Blood samples were then sent for genotyping at the Université catholique de Louvain AIDS reference laboratory in Belgium.

DNA extraction was performed from 200 µl of whole blood with the "Nucleospin® extraction kit (Macherey-Nagel, D-52313 Düren Neumann Neander Str. 6-8, Germany)" according to the manufacturer's recommendations. The extracted DNA was finally suspended in 100 µl of elution buffer.

All PCR assays were performed using a C1000 Touch™ Thermal Cycler (Bio-Rad 1000 Alfred Nobel Drive Hercules, California 94547 USA). Protease and reverse transcriptase region were amplified by nested PCR with the recombinant *Thermococcus kodakaraensis* KOD1 DNA polymerase enzyme (Merck, 2000 Galloping Hill Road Kenilworth, NJ 07033 U.S.A). A total of 4 primers (2 outer: MAW26 (5'-TGGAATGTGGAAAGGAAGGAC-3') and RT21 (5'-CTGTATTTCTGCTATTAAGTCTTTTGATGGG-3') for the first amplification and 2 inner: RT20 (5'-CTGCCAGTTCTAGCTCTG CTTC-3') and PRO1 (5'-CAGAGCCAACAGCCCCACCA-3') for the second amplification) were used¹¹⁰. These primers were used for all the amplifications independently of the PCR parameters.

All samples were amplified with a touch down procedure during the first amplification. After first amplification, a nested amplification was performed.

In case of three failures, the samples were amplified one more time without touch down during the first amplification. The second amplification was also a nested amplification.

Finally, remaining failing samples were amplified after cutting out the band of interest on a migration gel after second amplification (without touch down during the first amplification). After purification, the samples were amplified a third time with the same parameters as the second amplification. PCR parameters are described in table 1.

A 1,300-bp fragment containing the protease and the first 300 amino acids of the reverse transcriptase gene was generated. Before sequencing, samples were purified with the QIAquick kit (QIAGEN Sciences 19300 Germantown Rd Germantown MD 20874 USA). Amplification mixes are described in the two tables below. Notably, from the 200µl of whole blood used for DNA extraction, we obtained a solution of 100µl of extracted DNA. Only 1µl of this solution was used for the first amplification.

PCR parameters during first and second amplifications

	Number of cycles	Temperature (°C)	Time
First amplification	1	95	5 minutes
		95	30 seconds
	40	63 to 59*	20 seconds
		70	30 seconds
	1	4	∞
Second amplification	1	95	5 minutes
		95	30 seconds
	40	56	20 seconds
		70	27 seconds
	1	4	∞

* Touch down: 10 cycles at 63°C, 10 cycles with decreasing from 63 to 59°C and 20 cycles at 59°C.

First amplification

Components	Volume (μl)
Primer MAW26	0.75
Primer RT21	0.75
Water	10
KOD 1	12.5
DNA	1
Total	25

MAW26 (5'-TGGAAATGTGGAAAGGAAGGAC-3')

RT21 (5'-CTGTATTCTGCTAT TAAGTCTTTTGATGGG-3')

Second amplification

Components	Volume (μl)
Primer RT20	1.5
Primer PRO1	1.5
Water	20
KOD 1	25
DNA (from first amplification)	2
Total	50

RT20 (5'-CTGCCAGTTCTAGCTCTG CTTC-3')

PRO1 (5'-CAGAGCCAACAGCCC CACCA-3')

Sanger sequencing with seven primers* was performed with the Applied Biosystems® 3500 Genetic Analyzer (Life Technologies), following the AIDS laboratory protocol

Results were analyzed with the IDNS Smartgene software using the Stanford algorithm (HIVDB 8.4.0).

*(Pr3b: AGAGCCAACAGCCCCACCA, Pr4: GGGCCATCCATTCTGGCTT, PR2390: GATAGGGRGAATTGGAGGT, R5: GGGTCATAATACACTCCATG, RT3235 : GGATGRAGYTCATARCCCATCC, RT2882 : GGGNGAYGCATATTTTCARTWCC, RT2955 : CTAATYCCTGGYGTTCATTRTT).

V. Statistical analysis

This is a retrospective study including long-term follow-up and cross sectional data. Mean values were compared with the Student t-test. Proportions were compared with the Fisher-exact test. Differences in CD4⁺ means were also tested with the non-parametric Wilcoxon test, which resulted in p-values similar to the ones obtained with the Student t-test.

All the p-values presented in the future tables were calculated with one or two of the three methods mentioned above.

§2. Immunological outcomes

I. Results

Study population

From the 94 subjects described in the 2005-2008 study¹⁰⁹, 55 were included in the present research (23 females and 32 males). The remaining 39 patients were transferred to other centers and lost to follow-up (36/39) or died (3/39). The control group (LSC) included 106 subjects who initiated treatment after 2 years of age. Characteristics of both cohorts are described in table 3.

2014	ESC (55)	LSC (106)	p-value
Mean age at cART initiation	8.6 m (2.2m – 11.9m)	4,5 y (2.0y – 7.6y)	<0.001
Gender (Female)	23 (41.8%)	48 (45.3%)	0.86
Mean Age (years)	8.2 (6.9 – 10.2)	10,8 (6.0 – 14.6)	< 0.001
Mean time on cART (years)	7.6 (6.5 – 9.5)	6.0 (2.0 – 9.5)	< 0.001
Mother not caregiver	18 (32.7%)	53 (50.0%)	0.0045

*Table 3. Characteristics of ESC and LSC
In brackets: percentages or under and upper limit values*

Definitions

Children with CD4⁺% <15%, between 15-24% and ≥ 25% at treatment initiation were described respectively as low (LIS), intermediate (IIS) and high (HIS) immunologically responding subgroups.

First line therapies

In the ESC, 98% of children were initiated on cART with a regimen including one PI and two NRTIs and 2% with one NNRTI and two NRTIs.

In the LSC, 12% of children were initiated on cART with a regimen including one PI and two NRTIs (children between two and three years of age at treatment initiation) and 88% with one NNRTI and two NRTIs (children older than three years at treatment initiation) (Table 4).

PI/NNRTIs	NRTIs	ESC (55)	LSC (106)
Lopinavir/ritonavir	Stavudine/Lamivudine	50 (91%)	13 (12%)
Lopinavir/ritonavir	Zidovudine/Lamivudine	4 (7%)	/
Nevirapine	Stavudine/Lamivudine	1 (2%)	/
Efavirenz	Stavudine/Lamivudine	/	69 (65%)
Efavirenz	Abacavir/Lamivudine	/	23 (22%)
Efavirenz	Zidovudine/Lamivudine	/	1 (1%)

Table 4. First line therapies in the ESC and the LSC

Second line therapies

A large proportion of children have changed their treatment in both cohorts mainly due to revisions of the national recommendations. Due to its many side effects, d4T was replaced by another NRTI (ABC, AZT or TDF) in 76% (42/55) of the ESC and 53% (56/106) of the LSC.

In the ESC, 3 children (5%) initiated their treatment with AZT and 1 child (2%) with d4T, without any change during the entire follow-up period. One (2%) child changed from AZT to d4T due to anemia and 1 (2%) child changed from LPV/r to EFV with no specified reason.

In the LSC, 23 children (22%) initiated their treatment with ABC, 1 child (1%) with AZT and 10 children (9%) with d4T, without any change during the entire follow-up period. One (1%) child changed from EFV to LPV/r due to a skin rash.

Switch to a second line of cART due to VF was not significantly different between the ESC 7/55 (13.0%) and the LSC 15/106 (14.1%) ($p = 1$). Successive lines of treatment after VF are detailed in Tables 5 and 6.

Number of patients ESC	First line	Second line	Third line	Fourth line
1	d4T/3TC/LPV	d4T/3TC/EFV		
1	d4T/3TC/LPV	ABC/AZT/EFV		
1	d4T/3TC/LPV	ABC/AZT/EFV/LPV		
1	d4T/3TC/LPV	ABC/3TC/EFV		
1	AZT/3TC/NVP	AZT/3TC/LPV	ABC/3TC/EFV	
1	d4T/3TC/LPV	ABC/3TC/LPV	ABC/3TC/EFV	
1	d4T/3TC/LPV	ABC/3TC/EFV	AZT/3TC/LPV	TDF/3TC/LPV

Table 5. Second line therapies in the ESC

Number of patients LSC	First line	Second line	Third line	Fourth line
6	d4T/3TC/EFV	AZT/3TC/LPV		
1	d4T/3TC/EFV	AZT/ddI/LPV		
1	d4T/3TC/EFV	ABC/AZT/LPV		
3	d4T/3TC/EFV	ddI/AZT/LPV	AZT/3TC/LPV	
2	d4T/3TC/EFV	ddI/AZT/LPV	ABC/AZT/LPV	
1	d4T/3TC/EFV	ddI/AZT/LPV	TDF/3TC/LPV	
1	d4T/3TC/EFV	ABC/3TC/EFV	DDI/AZT/LPV	AZT/3TC/LPV

Table 6. Second line therapies in the LSC

Mean CD4⁺% in the ESC and the LSC: Cross sectional data

In 2014, there were significantly more children with CD4⁺% $\geq$ 25% in the ESC and significantly more children with CD4⁺% between 15 and 24% in the LSC. The rate of children with severe immunosuppression was comparable in both cohorts (Table 7)

2014	ESC (55)	LSC (106)	p-value
CD4 ⁺ < 15%	2 (3.6%)	6 (5.7%)	0.72
CD4 ⁺ 15-24%	2 (3.6%)	16 (15.1%)	0.033
CD4 ⁺ $\geq$ 25%	51 (92.8%)	84 (79.2%)	0.040

Table 7. Mean CD4⁺% in the ESC and the LSC in 2014

Mean CD4⁺% in the ESC and LSC: Longitudinal follow-up

Mean CD4⁺% at cART initiation was comparable and increased in the same proportions, in the ESC and the LSC during the first year after treatment initiation (+7.07% vs. +9.27%). After one year of treatment, mean CD4⁺% became and stayed higher in the ESC. This difference was not always significant (Fig 4, Table 8).

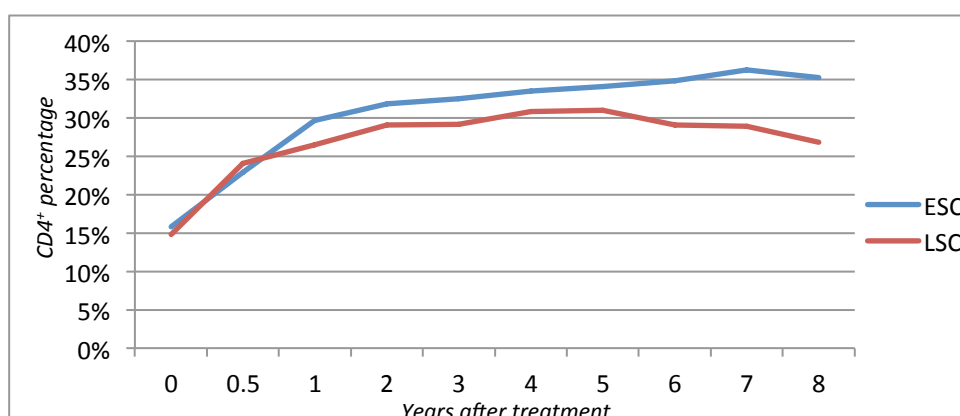


Figure 4. Mean CD4⁺% in the ESC and the LSC during the follow-up period.

Years after treatment initiation	ESC (Mean CD4 ⁺ %)	LSC (Mean CD4 ⁺ %)	p-value
0 (55/106)	15.80	14.78	0.46
0.5 (52/83)	22.87	24.05	0.49
1 (47/81)	29.64	26.50	0.048
2 (47/85)	31.83	29.06	0.092
3 (47/88)	32.45	29.11	0.034
4 (49/74)	33.47	30.80	0.092
5 (47/60)	34.09	30.98	0.039
6 (52/61)	34.77	29.07	<0.001
7 (33/37)	36.21	28.88	<0.001
8 (17/20)	35.18	26.82	0.0056

Table 8. Mean CD4⁺% in the ESC and the LSC during the follow-up period. Numbers in brackets represent the number of data available for that year.

All children included in the ESC (55/55) achieved normal CD4⁺% at least once during the entire follow-up period, contrary to the LSC where 86/106 (81.1%) children achieved normal CD4⁺% (p=0.0086).

In a second analysis, results were subdivided in three subgroups in function of the CD4⁺% at cART initiation (as explained in the definitions paragraph). First subgroup is the LIS, second is the IIS and third is the HIS. The proportion of children included in each subgroup was not significantly different in both cohorts (Table 9).

CD4 ⁺ % at cART initiation	ESC (55)	LSC (106)	p-value
LIS (CD4 ⁺ < 15%)	26 (47%)	61 (57%)	0.56
IIS (CD4 ⁺ 15-24%)	20 (37%)	33 (31%)	0.47
HIS (CD4 ⁺ ≥ 25%)	9 (16%)	12 (12%)	0.74

Table 9. Proportion of LIS, IIS and HIS in the ESC and the LSC

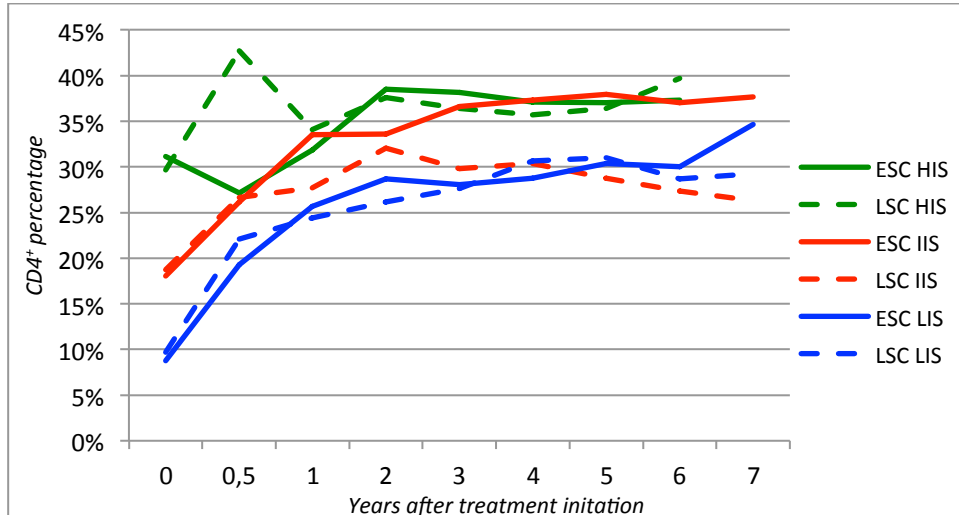


Figure 5. Mean CD4⁺ in the LIS, IIS and HIS from the ESC and the LSC during the follow-up period.

LIS from both cohorts had comparable mean CD4⁺ at cART initiation and during the entire follow-up period ($p>0.05$). Mean CD4⁺ became higher than 25% in both cohorts after two years of treatment (Fig 5 and Table 10).

Years after treatment initiation	LIS	ESC (55)	LSC (106)	p-value
0	(26/61)	8.77	9.70	0.31
0.5	(26/49)	19.27	22.12	0.72
1	(22/47)	25.68	24.39	0.50
2	(23/50)	28.70	26.19	0.23
3	(24/52)	28.08	27.61	0.78
4	(22/45)	28.77	30.62	0.34
5	(23/38)	30.39	30.98	0.73
6	(25/42)	30.00	28.71	0.11
7	(17/24)	34.65	29.26	0.029
8	(10/16)	32.00	26.28	0.11

Table 10. Mean CD4⁺ in the LIS from the ESC and the LSC during the follow-up period. Numbers in brackets represent the number of data available for that year.

In the ESC, 26/26 children achieved normal CD4⁺% at least once during the entire follow-up period and 47/61 (77%) children achieved normal CD4⁺% in the LSC (p=0.0081).

IIS from both cohorts had comparable mean CD4⁺% at cART initiation but in children from the ESC it became and stayed significantly higher than in the LSC, three years after cART initiation. Mean CD4⁺% became higher than 25% in both cohorts after two years of treatment (Fig 5 and Table 11). In the ESC, 20/20 children achieved normal CD4⁺% at least once during the entire follow-up period, and 27/33 (82%) children achieved normal CD4⁺% in the LSC (p=0.072).

Years after treatment initiation IIS	ESC (55)	LSC (106)	p-value
0 (20/33)	18.05	18.74	0.36
0.5 (18/27)	26.17	26.66	0.87
1 (19/25)	33.53	27.73	0.028
2 (18/28)	33.61	32.04	0.55
3 (17/28)	36.59	29.83	0.021
4 (18/25)	37.39	30.34	0.010
5 (16/16)	37.94	28.76	< 0.001
6 (18/16)	37.00	27.38	0.0027
7 (13/9)	37.69	26.33	0.0035
8....(7/4)	39.71	29.00	0.0072

Table 11. Mean CD4⁺% in the IIS from the ESC and the LSC during the follow-up period. Numbers in brackets represent the number of data available for that year.

HIS from both cohorts had comparable mean CD4⁺% at cART initiation and during the entire follow-up period (p>0.05) except at six months of treatment (27.1% vs. 42.7%, respectively). Mean CD4⁺% stayed higher than 25% in both cohorts during the entire follow-up period (Fig 5 and Table 12).

Years after treatment initiation HIS	ESC (55)	LSC (106)	p-value
0 (9/12)	31.11	29.67	0.49
0.5 (8/7)	27.13	42.71	< 0.001
1 (6/9)	31.83	34.11	0.65
2 (6/7)	38.50	37.57	0.82
3 (6/8)	38.17	36.40	0.59
4 (9/6)	37.11	35.72	0.70
5 (8/6)	37.00	36.43	0.90
6 (9/3)	37.33	39.67	0.38

Table 12. Mean CD4⁺% in the HIS from the ESC and the LSC during the follow-up period. Numbers in brackets represent the number of data available for that year.

Antituberculosis treatment in both cohorts

There was a higher rate of children treated with TB tri or quadritherapy during their follow-up period in the LSC, however on the numbers studied this difference was not significant.

During entire follow-up	ESC (55)	LSC (106)	p-value
TB tri or quadritherapy	26 (47%)	65 (61%)	0.096

Table 13. Tuberculosis tri or quadritherapy rates in the ESC and the LSC during the follow-up period.

II. Discussion

At the end of this chapter, we conclude that cART was highly effective to induce immunological restoration in both cohorts (ESC and LSC) and for the three subgroups (LIS, IIS, HIS). This underlines the importance of initiating cART in all children regardless of age and immunosuppression. In our study group, children included in the ESC had at least comparable (or even better) immunological outcomes than children included in the LSC supporting an early treatment initiation, as recommended by the international and the South African guidelines^{8,10,29,30,110}.

After comparison of cART regimens, children included in the ESC and the LSC initiated cART with 2 NRTI and 1 PI or 2 NRTI and 1 NNRTI according to age, which means that the guidelines for the first line therapies are clear and correctly applied. Second line therapies were more heterogeneous in both cohorts probably because different recommended options are available in the WHO¹¹¹ (e.g. LPV/r replaced by EFV, d4T+3TC replaced by ABC/ TDF+3TC) and the South African guidelines¹⁰ (e.g. LPV/r replaced by LPV/r + EFV or a third line, d4T + 3TC replaced by ABC + AZT). ddI is no more recommended in both guidelines but is still used in RLS. Finally, expert advice is recommended after first line failure with a LPV/r based treatment. Experts are not always available and the choice of treatment depends more often on the habits of the local staff.

At treatment initiation, the ESC and the LSC both had a high proportion (47% vs. 57%) ($p=0.56$) of children with severe immunosuppression (<15% CD4⁺%). This is consistent with a review from Koller et al. who reported 70% of severe immunosuppression in children <2 years of age who started treatment in low and middle-income countries in 2010¹¹². Surprisingly, patients included in the present study had comparable mean CD4⁺% independently of age at cART

initiation. It was not the case in European and West African studies^{76,113} who showed more severe immunosuppression when treatment was started later in life (-0.82% of mean CD4⁺% per year of cART initiation delay)⁷⁶. This difference could be explained in our study by the exclusion of rapid progressors¹¹⁴ who possibly died before 2 years of age (before cART initiation) in the LSC.

Influence of age at cART initiation, showed contradictory immunological outcomes. Four studies¹¹⁵⁻¹¹⁸ concluded the absence of influence of age at baseline and five studies^{76,78,113,119,120} described poorer immunological outcomes for late starters. The follow-up study of CHER⁷³ compared early time-limited cART with deferred cART in 377 South African infants with a median follow-up of 4.8 years. Early treated children (median age 7.4 weeks, CD4⁺% of 35%) had better clinical and immunological outcomes than children treated later (median age 20 weeks, CD4⁺% of 35%) even after planned treatment interruption at 40 or 70 weeks⁷⁸. Another follow-up study implemented in the same cohort showed the importance of an early identification and treatment of HIV-infected infants to stabilize CD4 counts at the highest levels possible. Once stabilized, children's CD4 counts appeared resilient, with good potential for recovery following treatment interruption⁷³. In our study, mean CD4⁺% was normal in both cohorts but there were statistically less children who achieved normal CD4⁺% in the LSC.

Yin et al. described a significant interaction between CD4⁺% and age on the probability of ever recovering a normal CD4⁺% within 4 years¹²⁰. With increasing age, baseline CD4⁺% had a stronger effect on the capacity to recover a normal CD4⁺%. Children included in our three subgroups achieved normal mean CD4⁺% independently of their immunological status at baseline. There were more children who achieved normal CD4⁺% in the ESC. This difference was significant for LIS but not for the IIS probably due to the small numbers in this second subgroup.

Finally, children from the ESC were, as expected, less often treated with TB tri or quadritherapy because they were younger at treatment initiation and at inclusion in the present study. However, this difference was not significant and treatments were initiated on a clinical basis, which could overestimate the real incidence of TB infection.

§3. Virological outcomes

A. Long term outcome in terms of viral load

I. Results

Study population

The 55 children from the ESC and the 106 children from the LSC were already described before (study population). First and second line therapies were also described in the immunological chapter.

Definitions

VF was defined as a VL above 1000 copies/ml in 2 successive blood samples taken at least 6 months apart.

High and low-level viremia were defined as a VL > 1000 copies/ml and between 50-1000cp/ml in one blood sample respectively.

Undetectable viremia was obtained when VL was less than 50 copies/ml.

Viral load in the ESC and the LSC: Cross sectional data

In 2014, children included in the ESC had significantly more undetectable VL. Children from the LSC had more low and high-level viremia and VF, but these differences are not significant (Table 14).

2014	ESC (55)	LSC (106)	p-value
VL < 50 cp/ml	48 (87.0%)	76 (71.7%)	0.029
VL 50-1000 cp/ml	4 (7.5%)	13 (12.3%)	0.42
VL > 1000 cp/ml	3 (5.5%)	17 (16.0%)	0.076
VL > 1000 cp/ml during 2 years	3 (5.5%)	13 (12.3%)	0.27

Table 14. Proportion of children with undetectable, low and high-level viral load and virological failure in the ESC and the LSC in 2014

Viral load in the ESC and the LSC: longitudinal data

At cART initiation, mean VL was significantly higher in the ESC (6.5 vs 5.7 log₁₀ cp/ml), but the difference waned 6 months after cART initiation. One year after cART initiation, the proportion of children with undetectable VL was comparable in both cohorts. The proportion of children with persistent undetectable VL from one year after treatment initiation until 2014 was also comparable.

As mentioned in the immunological chapter, switch to a second line of cART due to VF was not significantly different between the ESC 7/55 (13.0%) and the LSC 15/106 (14.1%) ($p = 1$). (Table 15)

	ESC (55)	LSC (106)	p-value
< 50 cp/ml 1 year after cART initiation	36 (65.5%)	75 (70.8%)	0.59
Always < 50cp/ml 1 year after cART initiation	19 (34.5%)	35 (33.0%)	0.86
At least once > 1000cp/ml after cART initiation	18 (32.7%)	43 (40.6%)	0.39
Switch 2nd line due to virological failure	7 (13.0%)	15 (14.1%)	1

Table 15. Virological outcome during the follow-up period

II. Discussion

At the end of this sub-chapter, we can conclude that cART was highly effective to control VL. Children from both cohorts had comparable virological outcomes with the exception, in 2014, of higher rates of undetectable VL in the ESC. This result was limited to that year and should be taken with caution. Taking together with the clinical, immunological and virological short-terms benefits of early cART, our long-term data encourage the recommendation to initiate cART early in life.

Some of the results presented in this sub-chapter can be underscored. VL was higher in the ESC at treatment initiation. This could be explained by their younger age and was already described in untreated perinatally infected infants, who had the highest HIV-1 RNA levels at 2 months of age followed by a slow decline during the first two years of life. These high VL and slow decline reflect the lower efficiency of the immature immune system, which cannot contain the viral replication and has possibly a greater number of HIV-susceptible cells¹²¹.

One year after treatment initiation, 65 to 70% of the infants included in both cohorts achieved undetectable VL. These rates are in the same range than others have described in previous studies implemented in RLS and developed countries (50-85%)^{24,75,122-124}. The observed differences between 50 and 85% may be due to different patients specificities (cART, HIVDR, country of origin...).

Approximately one third of the children included in both cohorts sustained undetectable VL during the entire follow-up period.

Finally, the proportion of children who experienced VF during the follow-up period and switch to a second line therapy was comparable in both cohorts (13.0% vs 14.1%). This is consistent with two other studies including respectively 1692 and 5485 South African children in 2011 (9 and 19%)^{87,88}.

B. Resistances in the long term.

I. Results

Study population

From the 161 childrens' blood samples included in the immunological chapter, 93 proviral DNAs were successfully genotyped: 29/55 (52.7%) in the ESC and 64/106 (47.3%) in the LSC.

In these subgroups, children included in the ESC were as expected younger at treatment initiation and at study implementation in 2014. They had a longer duration of follow-up. Sex ratio and percentage of children having their biological mother as caregiver, was comparable in both cohorts (Table 16).

2014	ESC (29)	LSC (64)	p value
Mean age at treatment initiation (months)	9.3 (3.1 – 11.4)	54.0 (24.0– 91.2)	<0.001
Gender (Female)	12.0 (41.4%)	32 (50.0%)	0.51
Mean age in 2014 (years)	8.3 (6.9 – 10.2)	10.5 (6.4 – 14.6)	<0.001
Mean time on cART (years)	7.6 (6.6 – 9.4)	6.0 (2.0 – 9.1)	<0.001
Mother not caregiver	9.0 (31.1%)	33 (51.6%)	0.076

Table 16. Sequenced patients characteristics during the follow-up period

Both cohorts were treated with a NRTI backbone (two drugs) and one PI in the ESC or one NNRTI for most children in the LSC. Five children (7.8%) aged between 2 and 3 years included in the LSC, started their treatment with a PI based regimen like recommended by the South African guidelines (Table 17).

PI/NNRTIs	NRTIs	ESC (29)	LSC (64)
Lopinavir/ritonavir	Stavudine + Lamivudine	27	5
Lopinavir/ritonavir	Zidovudine + Lamivudine	2	/
Efavirenz	Stavudine + Lamivudine	/	44
Efavirenz	Abacavir + Lamivudine	/	15

Table 17. cART regimens in the sequenced patients from both cohorts at treatment initiation

Switch to a second line due to VF was not significantly different between the ESC 4/29 (13.7%) and the LSC 8/64 (12.5%) ($p = 1$). One child changed from therapy due to side effects in both cohorts. Treatments in 2014 are described in Table 18.

PI/NNRTIs	NRTIs	ESC (29)	LSC (64)
Lopinavir/ritonavir	Abacavir + Lamivudine	21	6
Lopinavir/ritonavir	Zidovudine + Lamivudine	3	5
Lopinavir/ritonavir	Tenofovir + Lamivudine	1	1
Lopinavir/ritonavir	Abacavir + Zidovudine	/	2
Efavirenz	Abacavir + Zidovudine	1	/
Efavirenz	Abacavir + Lamivudine	2	42
Efavirenz	Tenofovir + Lamivudine	/	1
Efavirenz	Stavudine + Lamivudine	/	7
Efavirenz + Lopinavir	Abacavir + Zidovudine	1	/

Table 18. Treatment in the sequenced patients from both cohorts in 2014

Definitions

Undetectable viremia: < 50 copies/ml

Low-level viremia: 50 – 1000 copies/ml

High-Level viremia: > 1000 copies/ml

Virological failure: > 1000 copies/ml in 2 successive blood samples taken at least 6 months apart

High-level HIVDR: was defined by the IDNS Smartgene software with the Sandford algorithm. It is a mutation (or a set of mutations) that confers a high-level resistance to an antiretroviral drug. It is important to notice that some mutations may confer high-level resistance to one drug and low or intermediary resistance to others.

Viral load and viral resistances

In 2014, children from the ESC and the LSC had comparable virological outcomes in terms of percentage having an undetectable VL, a low or a high-level viremia, and both cohorts share comparable rates of VF. Children from the ESC had a significantly higher rate of high-level HIVDR but also a lower proportion of children with no proviral mutations detected (Table 19).

	ESC (29)	LSC (64)	p-value
Undetectable VL	22 (75.8%)	43 (67.2%)	0.47
Low-level viremia	4 (13.8%)	6 (9.4%)	0.50
High-level viremia	3 (10.4%)	15 (23.4%)	0.17
Virological failure	4 (13.7%)	10 (15.6%)	1
High-level HIVDR	16 (55.2%)	14 (21.9%)	0.032
No proviral mutation	13 (44.8%)	45 (69.2%)	0.023

Table 19. Virological outcomes in the sequenced patients from both cohorts in 2014

Children from the ESC more often presented any NRTI or NNRTI mutation. There was no difference for PI mutation rates between both cohorts. Children from the ESC had higher rates of NRTI and NNRTI high-level HIVDR, but this difference was not significant. Proportion of children with multiclass high-level HIVDR was comparable in both cohorts (Table 20).

	ESC (29)	LSC (64)	p-value
Any NRTI mutation	10 (34.5%)	9 (14.1%)	0.030
NRTI high-level HIVDR	9 (31.0%)	9 (14.1%)	0.087
41L	1	1	0.53
67N	0	1	1
70R	0	1	1
74I	1	0	0.31
115F	1	0	0.31
184I/V	9	9	0.087
215Y	0	1	1
219E/219N	1	1	0.53
TAMs	2	4	1
Any NNRTI mutation	13 (44.8%)	13 (20.3%)	0.024
NNRTI high-level HIVDR	9 (31.0%)	11 (17.2%)	0.17
98G	0	3	0.55
103N	2	8	0.72
106M	2	2	0.59
108I	0	2	1
138A	2	0	0.095
179D	2	0	0.095
181C	5	1	0.01
188C/H	1	1	0.53
190A	0	2	1
221Y	0	2	1
225H	0	3	0.55
227L	1	0	0.31
230	1	0	0.31
Any PI mutation	3 (10.3%)	9 (14.1%)	0.75
PI high-level HIVDR	1 (3.4%)	2 (3.1%)	1
15V	1	0	0.31
30N	1	0	0.31
33F	0	1	1
36I	1	0	0.31
46L/I	0	6	0.17
50V	0	1	1
54V	0	1	1
58E	0	1	1
69K	1	0	0.31
73S	1	1	0.53
74S	1	0	0.31
82A/T	0	2	0.55
83D	1	0	0.31
Multiclass high-level HIVDR	3 (10.3%)	7 (10.9%)	0.75

Table 20. Proviral mutations in the sequenced patients from both cohorts in 2014

In the ESC and the LSC, the most frequent NRTI mutation was 184V/I and the most frequent NNRTI mutations were 181C and 103N respectively. In the LSC, 46L was the most frequent PI mutation (Table 21).

Among the 29 children who initiated their treatment with a PI based regimen in the ESC, 13 presented a virus with a NNRTI HIVDR and 9 had a virus with high-level HIVDR. Interestingly, only 2 of these 9 children (22%) with high-level HIVDR were treated with NNRTI in a second line therapy. In the LSC, 9 children had a PI HIVDR and 2 a high-level HIVDR. One of these 2 children was treated with PI in a second line therapy.

Among children that switched to a second line therapy due to VF, 3/4 (75%) children in the ESC and 2/8 (25%) children in the LSC presented high-level HIVDR to at least one component of their treatment. The remaining 1/4 and 6/8 switched to a second line therapy without any high-level HIVDR.

II. Discussion

Management of HIVDR in adults and children is a growing global health concern. Prevention, monitoring and response to HIV drug resistances are essential to achieve the UNAIDS 90-90-90 targets for treatment in 2020. These targets include diagnosing 90% of people with HIV infection, providing treatment to 90% of those diagnosed and ensuring that 90% of people on treatment achieve virological suppression.

Since 2010, many efforts were made in SA to improve access to diagnosis and to treat HIV infection as early as possible in infancy. Benefits of early treatment initiation on infant mortality^{18,34,125}, neurodevelopmental outcome¹²⁶, growth recovery¹²⁷, immunologic restoration and virological suppression^{69,70}, have been demonstrated in short term follow-up studies implemented in RLS.

Knowing the major benefits of an early treatment initiation in infants and the growing efforts to diagnose HIV as early as possible in life, most of the future HIV infected children should initiate treatment in infancy.

Our results monitor HIVDR in children living in a RLS of SSA. Children treated before one year of age with a PI based regimen, as recommended by the international guidelines, had good virological outcomes with at least comparable rates of undetectable VL, low and high-level viremia and VF than children treated after two years of age with a NNRTI based regimen. Despite this favorable outcome, children from the ESC had surprisingly higher rates of NRTI and NNRTI HIVDR.

Higher NRTI mutations rates in the ESC may be explained by different factors.

Children from the ESC were dependent on their caregiver to take their treatment during a longer period of time because they initiated cART earlier in life. The mother was not the caregiver for many children in the ESC and the LSC (31.1% vs 48.4% respectively), which implies the involvement of another caregiver. As mentioned in, the introduction caregiver's age, biologic relationship with the child, HIV status and adherence to their own treatment may influence the success of the child's treatment³⁷⁻⁴². Presence of a unique caregiver⁴³ is associated with better adherence.

Treatment initiation early in life is associated to other risk factors of suboptimal cART administration. Emesis is a common cause of under dosing for infants because re-dosing is not always done¹²⁸. Low-level drug concentration in the child's blood due to rapid growth³⁹, drug interaction with rifampicin⁴⁷ or inadequate intake due to poor drug palatability or unadapted formulations is involved^{48,49}. Finally, socio-economical restrictions like transport costs to the medical center or the need for a fridge to keep syrups cool may also lead to VF.

Despite being younger in 2014, children from the ESC had a longer duration of therapy, which is associated with decreased adherence and increased risk of resistance¹²⁸. Moreover, infants and young children tend to be maintained a longer time than adults on failing regimens because of difficulties with adherence and limited treatment options⁴⁶. Moving into adolescence is also associated with poor adherence^{129,130} and could be a risk factor of VF in the LSC where 23 children (36%) were more than 12 years old in 2014.

Higher NNRTI mutation rates in the ESC could most simply be explained by the presence of resistant virus, acquired from their mother or during PMTCT, before cART initiation. Children born from HIV infected mothers have normally benefited from a PMTCT with NVP. In case of failure of this prevention, infants are at higher risk to develop NNRTI resistances¹³⁰⁻¹³². Persistence of this resistance during the next years could lead to treatment failure in case of NNRTI based treatment. For this reason, children in the ESC initiated their treatment with a PI, while this was rarely the case in LSC. Pre-treatment resistances were certainly present in both cohorts. Among the 9 patients with high-level NNRTI HIVDR in the ESC, only 2 (22%) were treated with a NNRTI after PMTCT. The 7 others acquired a resistant virus from their mothers or during the PMTCT. NNRTI pre-drug resistance (PDR) is dramatically increasing in RLS. A recent meta-analysis about pretreatment HIVDR in children living in SSA described a rate of 42.7% of PDR among PMTCT-exposed children and 12.7% among PMTCT-unexposed children¹³³.

In contrast to our opinion, some authors stated that viral resistance selected by prior exposure to single-dose NVP might wane with time and allow switching to a NNRTI based regimen¹³⁴. Consequently, some studies have pleaded for a switch to a NNRTI based regimen in children virologically suppressed under PI based regimen¹³⁵⁻¹³⁷ due to reduced treatment costs, less metabolic toxicities and limited second line options. The NEVEREST 3 study¹³⁷ conducted in

Johannesburg, SA, from 2010 to 2013, enrolled 300 HIV-infected children exposed to NVP for PMTCT who were aged 3 years or older and had plasma HIV RNA of less than 50 cp/ml during LPV/r therapy; 292 (98%) were followed up to 48 weeks after randomization. Participants were randomly assigned to switch to EFV-based therapy (n=150) or continue LPV/r-based therapy (n=148). The probability of VF was comparable in the EFV group (2.7%) and in the LPV/r group (2.0%). By 48 weeks, CD4⁺% was 2.88% (95% CI, 1.26%-4.49%) higher in the EFV group than in the LPV/r group. In this study, among HIV-infected children exposed to NVP for PMTCT and with initial viral suppression with LPV/r therapy, switching to EFV-based therapy compared with continuing LPV/r therapy did not result in significantly higher rates of VF.

We couldn't make this recommendation because in our study, switching from a PI to a NNRTI based regimen could have led to VF for at least 7/29 (24%) children included in the ESC, due to presence of persistent high-level HIVDR. Long-lived NNRTI-resistant viruses were also described in a prospective South African Study including children who failed HIV-prophylaxis. NNRTI-resistant HIV persisted in 26/27 (96%) of children with a median age of 21 month, which suggests that resistance will likely persist through 36 months of age, when children qualify for NNRTI-based ART¹³⁸. Our results, added to those of other studies^{136,139}, encourage a close monitoring of VL in PMTCT-exposed and unexposed children when switching therapy. The current first-line ART regimen with a NNRTI could also be revised for children older than 3 years. Indeed, 24% of children included in ESC had resistance to NNRTIs despite having never been exposed to this drug in their treatment, with the exception of a possible PMTCT (PDR). The WHO recommends countries to consider changing their first-line regimens if levels of PDR reach 10%⁴⁴.

The switch to a second line of therapy because of VF, occurred in 25% of children from the ESC and 75% children from the LSC without any HIVDR. This highlights the difficulties to assess adherence of children despite the presence of experienced counselors. In case of recurrence of VF, most of these children will not be able to access a third line therapy due to the unavailability of the newest drugs in RLS. Genotyping allows assessing the need to switch to another treatment in case of first or second line failure in children with suspected suboptimal adherence. Decentralized genotypic resistance testing at least at provincial level and training of clinicians to interpret results of genotyping is an increasing need in RLS⁹⁵.

After analysis of proviral mutation prevalence, 18/93 (19.4%) children harbored the 184V NRTI mutation, 11/93 (11.8%) the 103N NNRTI mutation and 2/93 (2.1%) harbored the 82A PI mutation. The 184V mutation is selected by 3TC/FTC and reduce susceptibility to these drugs more than 100-fold. They are also selected by and cause low-level resistance to ABC and ddI and slows the emergence of AZT, d4T, and TDF resistance. The 103N mutation is selected in patients receiving NVP and EFV. It reduces NVP and EFV susceptibility by about 50 and 20-fold, respectively. In 2004, Pao et al. studied the persistence of 103N and 181C mutations during the 3 years after baseline genotyping in 14 children. 103N persisted during the entire follow-up period in contrast to 181C, which disappeared after 25 months¹⁴⁰. This was not the case in our study, where 181C persisted in five children from the ESC despite they didn't receive NNRTI since cART initiation. 82A is a mutation selected primarily by IDV and LPV and reduces susceptibility to these PIs. 82A is associated with cross-resistance to ATV and NFV. It is also associated with reduced susceptibility to SQV and FPV when it occurs in combination with additional PI-resistance mutations.

Another study published in 2014, about HIVDR in children at VF in the KZN⁹¹ also described 184V and 103N as the most prevalent NRTI and NNRTI mutations. Only 1/89 (1.1%) child had one major PI-resistance (82A). This low rate of PI resistances probably explains the comparable virological outcomes in the ESC and the LSC despite a higher rate of NRTI and NNRTI in the ESC. Previous studies demonstrated the effectiveness of LPV/r in monotherapy to treat HIV infected people even after first-line cART failure in RLS^{131,131,141-143}. In the EARNEST study, 78% of HIV infected patients had <400cp/ml after 144 weeks with LPV/R monotherapy¹⁴². However, LPV/r monotherapy demonstrates lower rates of virological suppression when compared with LPV/r in tritherapy and therefore should not be considered as a preferred treatment option for widespread use in antiretroviral-naïve patients or for second line-therapies¹⁴¹⁻¹⁴³.

We described genotyping as a tool to assess adherence in case of virological failure. Other possibilities such as the measurement of ARV concentration in patient's hair are available. LPV/r hair concentration was considered as a strong predictor of viremia in Asian and African HIV-infected children¹⁴⁴⁻¹⁴⁶. But, ARV concentration in hair was measured by liquid chromatography/tandem mass spectrometry, which is not available in most of RLS. Moreover, this method doesn't allow to look for viral mutations and guide health authorities for the implementation of the different lines of treatment.

§ 4. Limitations

The three chapters and subchapters about CD4⁺%, VL and HIVDR from this retrospective cohort study share some limitations.

Selection bias

The retrospective nature of the study excluded all dead or lost to follow-up children between treatment initiation and the present study. Data collected at baseline could not be representative of all the infants from this region. This could explain the comparable value of mean CD4⁺% in both cohorts at treatment initiation.

Rapid progressor infants died before initiation of treatment in the LSC, which could improve the immunological and virological results in this cohort.

We couldn't genotype all the blood samples collected in both cohorts. Some proviral DNAs could be genotyped during the first attempt despite a very long duration of undetectable VL and other never could be genotyped despite a shorter duration of undetectable VL. But surprisingly, numerous amplifications succeeded really well after two or three attempts despite an unchanged amplification technique. One explanation could be the small volume of DNA (1µl) amplified after extraction. Maybe the proportion of proviral DNA was different during the different attempts due to a lack of homogeneity of the DNA concentration in the 100µl of extracted DNA. Another explanation could be linked to operator variability during the manipulations despite all precautions. Whatever the reason, we couldn't genotype 42% of all the samples which represents a risk factor to select some samples which are more easily sequenced.

Information bias

Data were collected in the medical files recorded on paper for most of them. There was a risk of mistake at transcription during the years of follow-up and during the present study.

Some data were missing in the medical file because they were not written by the medical staff or because there was no blood sample taken that year. This could increase the rate of children with persistent undetectable VL during the entire follow-up period.

There was a decreasing number of data available over the years because all children were not followed-up for the same number of years, which affected the power of the studies.

Confusion bias

Children included in the ESC and the LSC had, for most of them, different cART regimens (PI vs NNRTI) at treatment initiation. Our conclusions could be different if PI or NNRTI-based regimen were used in both cohorts. We couldn't make multivariate analysis for age and treatment because these two variable were inseparable for most of our patients. We cannot know for sure if the observed differences are due to early treatment or to differences in cART associations.

For the analysis of resistances, we could not determine if children received PMTCT or were infected with a resistant virus from their mothers, because we had no pre-treatment viral genotyping available. We only conclude that NNRTI PDR were present in children never treated with NNRTI during their

cART. The real rate of PDR could be an underestimate, because we couldn't assess the rate of NNRTI PDR in children who had NNRTI as part of their cART.

The reason why the LSC had less NNRTI HIVDR is not known for sure. Children from the LSC were less often treated by their mothers in 2014. We could suppose that these mothers died due to HIV infection. Probably a high proportion of these mothers didn't know their HIV positive status during pregnancy and delivery. This could explain a lower proportion of PMTCT in the LSC and why this cohort had less NNRTI HIVDR.

We concluded that children treated with a second line therapy without HIVDR detected at genotyping, had no resistance and didn't take their treatment. But, the decrease of resistant variants after a long period without treatment could also explain why we didn't find resistances in some children with VF. We could only detect the re-emerging wild type virus.

Finally, we detected proviruses because most of the children had undetectable VL. The proviruses represent all the viral genomes included in the human cells but not all replicative viruses, which are better detected with viral RNA. Proviruses HIVDR are interesting from an epidemiological point of view to assess the risk of HIVDR in the general population before VF occurs and at an individual level to avoid wrong therapeutic choices. In case of VF, viral RNA should be used at an individual level.

III. HIV and Hepatitis **B virus co-infection in** **children**

I. Introduction

Among the estimated 35 million people affected with HIV worldwide¹⁴⁷, approximately 2–4 million are chronic carriers of HBV¹⁴⁸. HIV/HBV coinfection worsens the natural progress of HBV infection because it decreases the chance to clear acute HBV infection, induces faster progression to cirrhosis and a higher risk of hepatocarcinoma¹⁴⁹. Early initiation and better access to cART has allowed more children to reach adulthood, however chronic carriage of HBV constitutes a major risk factor of morbidity and mortality due to hepatic complications¹⁵⁰.

Since the implementation of hepatitis B vaccination in the Expanded Program of Immunization (EPI) in 1995, the apparent prevalence of chronic HBV infection decreased from 10.4% to 5–7%¹⁵¹ in South African children. In 2001, evidence for complete elimination of HBs antigen (HBsAg) carriage has been reported in children less than 5 years of age with unknown HIV status¹⁵². To our knowledge, since 2000, only two studies described the prevalence of chronic HBV (1.2–1.7%) in HIV-infected children living in SA. Gauteng and North West provinces were mainly represented, with only a few patients who came from other provinces (Mpumalanga, Limpopo, and Northern Cape)¹⁵³.

Since its initiation in SA, hepatitis B vaccination coverage rates increased from 50% to 79% in the general population in 2011¹⁵⁴. However, HIV-infected children do not respond adequately to the routine immunization schedule due to a weaker immune system and no restoration of the HBV immunity after immune reconstitution¹⁴⁹. Due to the absence of national HBV serosurvey in SA, data on the long-term impact of the vaccine on HIV-infected children are scarce.

Finally, HIV has a dramatic impact on families. Many children become orphans, are critically ill and their caregivers often ignore the patients' history. Record of vaccinations is often unreliable and not reflected by serological results.

The survey that we performed first aims to describe the prevalence of HIV/HBV co-infection in children living in a RLS of the KZN province. Secondly, to compare the HBV vaccination response in HIV infected and uninfected children from this same region.

II. Methodology

Study Procedures

Children between 5 and 15 years of age were included on the basis of their HIV status just before their consultation with the local medical staff. Ten with uncertain HIV serological status were verified and were negative. Parents or caregivers of all subjects gave a written informed consent prior to any procedure. A questionnaire was filled with the investigator and blood samples were taken. Caregivers had to mention vaccination in infancy without indicating the number of administered doses. The study was approved by the South African ethical committee «Umgungundlovu Health District Review Board (UHERB)» and the Belgian Ethical committee «Comite d’Ethique Hospitalo-Facultaire SaintLuc Universite Catholique de Louvain».

Study Population and Setting

This survey was conducted in SA between September and December 2014 in the Edendale Regional Hospital located in a peri-urban area and RLS setting of the KZN province. The overall prevalence of HIV in the KZN province is the highest of SA with 16.9% of people infected¹⁵⁵. More than 3.000 patients (in majority mothers and their children) are currently on follow-up in a HIV family clinic built within the hospital complex.

One hundred and eighty-six HIV-infected children; 84 females (45.1%), with a median age of 9.1 years (range 6.5–14.8 years) were included in this study during their routine consultation at the HIV family clinic. Patients were distributed in two subgroups: the 5–10 years (103 children) and the 11–15 years (80 children). Three patients were excluded from the study due to technical failures during the laboratory analysis.

One hundred and seventeen HIV-uninfected children; 44 females (37.6%), with a median age of 9.0 years (range 5.8 and 15.0 years) were enrolled during their consultation at the emergency service of the hospital. Same distribution was done as in the other cohort: the 5–10 years (74 patients) and the 11–15 years (34 children) subgroups. Nine patients were excluded due to technical failures.

Laboratory Procedures

Blood samples were collected and sent to an external laboratory (Global Lab Durban, Amanzimtoti, South Africa). HBV serology including anti-HBs antibodies (anti-HBs), HBsAg and anti-HBc antibodies (anti-HBc) was performed by ADVIA Centaur XP immunoassay system (Siemens Healthcare GmbH, Henkestraat 127, 91052 Erlangen, Germany) with dedicated reagents following the manufacturer's recommendations.

Statistical Analysis

Proportions between HIV infected and uninfected cohorts were compared using the Fisher exact P-value test to assess the significance of difference between the two cohorts. Chi-square trend test was used to study the significance of differences between the age subgroups in the two cohorts.

Definitions

Ongoing HBV infection: isolated positive HBsAg.

Past HBV infection: positive anti-HBc with anti-HBs.

Vaccinated against HBV: isolated detection of anti-HBs (≥ 10 IU/L)

III. Results

Serological Markers of Past or Present HBV Infection

Non-significantly higher rates of HBV infection were found in the HIV-infected compared to the HIV-uninfected cohort ($P=0.3$). Prevalence of HBV infection did not increase with age in both cohorts ($P=0.8$ for HIV-infected and not calculable for HIV-uninfected cohorts) (Table 21).

	HIV-infected			HIV uninfected		
	5-10 years	11-15 years	Total	5-10 years	11-15 years	Total
Ongoing infection	0/103 (0%)	1/80 (1.3%)	1/183 (0.5%)	0/74 (0%)	0/34 (0%)	0/108 (0%)
Past infection	2/103 (1.9%)	1/80 (1.3%)	3/183 (1.6%)	0/74 (0%)	0/34 (0%)	0/108 (0%)

Table 21. Serologic Markers of Past and/or Ongoing Infection in the HIV-Infected and Uninfected Cohorts

Serological Evidence of Immunity

Prevalence of isolated anti-HBs ($\geq 10\text{IU/L}$) was lower in HIV-infected than in HIV-uninfected children ($P < 0.001$). This was observed in both age subgroups (5–10 and 11–15 years) ($P < 0.001$). The HIV- infected cohort had a significant lower immunity in the 11–15 years subgroup ($P = 0.035$) (Table 22).

	HIV-infected			HIV uninfected		
	5-10 years	11-15 years	Total	5-10 years	11-15 years	Total
Presence of anti-HBs	21/103 (20.4%)	8/80 (10.0%)	29/183 (15.8%)	49/74 (66.2%)	17/34 (50.0%)	66/108 (61.1%)

Table 22. Comparison of the Immunity Against HBV in the HIV-Infected and Uninfected Cohorts According to the Age Subgroup of the Patients

Evidence for Vaccination Against HBV According to the Road to Health Cards (Vaccination Card or RHC) and the Caregivers

In the HIV-infected cohort, 7/183 (3.8%) caregivers brought a RHC with proof of vaccination and 6/7 (85.7%) had negative anti-HBs. In the HIV-uninfected cohort, 51/108 (47.2%) caregivers brought a RHC with proof of vaccination but 20/51 (39.2%) had negative anti-HBs despite records showing they had been fully vaccinated (three doses of vaccine). Parents and caregivers of HIV-infected patients asserted that 138/183 (74.6%) of the children were vaccinated in infancy versus 99/108 (91.7%) in the HIV-uninfected cohort. The other caregivers did not remember if their child was vaccinated in infancy.

IV. Discussion

Despite a theoretical higher risk to develop chronic HBV, the 183 HIV-infected children included in this study had low rates of past and ongoing infections. No HIV-uninfected child had evidence of HBV carriage. Knowing that most of HBV infections are contracted before 5 years of age in Africa¹⁴⁹, these results are encouraging. A low rate of HBsAg (1.7%) was also found in 60 HIV-infected children between 1 and 16 years of age living in two other provinces of SA¹⁰⁷. Previous studies in children younger than 5 years, showed ranges from 0.4% in children with unknown HIV status¹⁵⁶ to 2.7% in HIV-infected children¹⁵³.

Prevalence of anti-HBs was significantly lower in HIV-infected (15.8%) versus uninfected children (61.1%). The rate of seroconversion was comparable with another North American study implemented in HIV-infected adults (17.5%) where plasma HIV RNA level of <400 copies/mL at the time of vaccination was associated with a protective antibody response.

Immunity was not checked and no extra dose was administrated after administering the three doses of vaccine at 6, 10, and 14 weeks in the HIV cohort. The standard immunization schedule was already shown to be suboptimal in immunosuppressed children¹⁵⁷⁻¹⁵⁹ and repeated or increased doses of vaccine after immune recovery may be considered¹⁵⁹. Ideally, anti-HBs should be measured 4–8 weeks after the last vaccine dose and one to three further doses should be administrated in case of vaccine failure (Ab titer <10 IU/L). Immunocompromized subjects should be tested annually and revaccinated if anti-HBs <10 IU/L^{158,160}.

HIV-uninfected subjects included in the present study presented low serological rates (61.1%) of anti- HBs, even when they had a full vaccination schedule completed in their RHC. It tends to confirm waning of serological markers more than a lack of vaccination in this cohort. It is a well-documented phenomenon that does not necessarily imply a loss of protection against HBV¹⁶¹. HIV- uninfected children from other South African provinces had higher rates of anti-HBs (81.7%) with a clear pattern of waning immunity and rising HBV chronic carriage with increasing age. This was an argument to consider hepatitis B vaccine booster dose for pre-adolescents¹⁰⁷. We could not confirm rising of chronic HBV carriage with age in HIV-uninfected children due to the absence of infection in both age subgroups.

Parents or caregivers reported a high rate of vaccination, in both HIV-infected and uninfected children (74.6% and 91.7%, respectively) despite the low prevalence of anti-HBs in both groups. These reported results are compatible with a study which found 97%, 92%, and 74% vaccination rates at 6, 10, and 14 weeks in the province of KZN¹⁶².

Because they were included in the study during routine consultation and as many caregivers were not the parents, only a few caregivers of HIV-infected children brought the RHC to the consultation.

This study has some limitations. It is a retrospective study limited to a single clinic with only 291 included children. A larger survey at regional or national levels would be of great interest. The origin and composition of the used vaccines were unknown and may be different overtime with potential impact on immunogenicity. Some caregivers did not remember if their child was vaccinated in infancy, which represents a recall bias. Finally, the site of injection and the storage methods of the vaccines were not specified, which could also influence the effectiveness of the vaccination¹⁶².

IV. Discussion

I. Ending the AIDS epidemic by 2030

There is a global consensus that the opportunity now exists to end the HIV epidemic. HIV treatment, condom promotion, behavior change, male circumcision and programs with key populations have clearly demonstrated their capacity to sharply lower the rates of new HIV infections¹⁶⁴.

Convinced by the efficacy of these procedures, the UNAIDS introduced the ambitious 90-90-90 and 95-95-95 targets for treatment in 2020 and 2030 respectively¹⁶⁴. Diagnosing people with HIV infection, providing treatment to those diagnosed and ensuring that people on treatment achieve virological suppression are the three main axes to control the epidemic.

To achieve these ambitious targets, a rapid scale up of essential prevention and treatment approaches are needed. Without fast-track strategy, the AIDS epidemic will continue to outrun the response, increasing the long-term need for HIV treatment, and increasing future costs¹⁶⁴.

Specific prevention and therapeutic programs were implemented in infants and children to achieve the UNAIDS targets. PMTCT by cART is one of the WHO priorities with 76% (60-88%) of all HIV pregnant women receiving cART in 2016¹⁶⁵. However, even with continued progress in PMTCT, WHO and UNICEF project 1.9 million children will require HIV treatment in 2020¹⁶⁵.

For these HIV-infected children early diagnosis is essential, but only 40% of them received early infant diagnosis in 21 priority countries in 2012¹⁶⁵. HIV testing should therefore be proposed at any age especially for malnourished children or those infected with TB¹⁶⁶⁻¹⁶⁸.

The latest international recommendations for the treatment of HIV-infected infants are expected to increase the proportion of early treated children during the next years. Assessment of the long-term effectiveness and efficiency of

an early cART initiation is essential, especially in places with high HIV prevalence and poor resources, to reach the UNAIDS 95-95-95 targets in 2030.

II. Ambitious targets for ambitious people living with limited resources

From an unequal distribution of HIV to an equal distribution of cART

As explained before, the world is embarking on a fast-track strategy to end the HIV epidemic within 12 years. After three decades of fight against the virus, we are entitled to ask ourselves if the UNAIDS targets are realistic, especially in a resource limited context.

Increasing access to cART is expensive and we could rightly fear that some governments will have to make choices between HIV prevention programs, HIV treatment programs and other health programs (malnutrition, tuberculosis, malaria...).

In SA, all treatments expansion scenarios based on higher CD4 thresholds for treatment initiation have been estimated to generate simultaneous health and economic benefits. The most substantial benefits occur when treatment is available to all people living with HIV, regardless of CD4 count. Rapid expansion of HIV treatment to all people living with HIV is projected to avert 3.3 million new HIV infections in SA through 2050 and save US\$30 billion¹⁶⁹. Even in SA, where more people living with HIV than any other country, estimates indicate that the country would reach the financial break-even point within a decade after scaling up treatment to all people, regardless of CD4 count^{169,170}.

All the optimistic forecasts cited before will only be achieved, on the condition that the HIV treatments remain effective on the long-term. This will be obtained by increasing adherence to the treatment and regular surveys for drug susceptibility. In case of failure, alternative cART should become available.

Improve adherence to cART

As in other chronic illnesses, adherence is often a great challenge especially in infants and adolescents. WHO has identified key research and development priorities to improve treatment of HIV infected children. Developments of age-appropriate fixed-dose combinations (FDCs) that include promising new antiretroviral drugs, such as DTG or TAF are central¹⁷¹. In 2016, the European Medicine Agency (EMA) published a report on pediatric development of FDCs for the treatment of HIV infection. The experts insisted on some drug characteristics that could improve adherence. Drugs to prioritize should have a high tolerability and safety profile, suitable age appropriate formulations and a good palatability. They should be administrated once daily without special dietary requirements. A high barrier to resistance may also be considered although it is not absolutely critical. These drugs have to be adapted for infants because they have potentially the greatest need. In RLS, the availability of FDCs for all pediatric age groups would also facilitate prescribing, which may increase particularly the youngest children's access to care¹⁷².

Different strategies to simplify drug administration have been developed. Short cycle therapy has been initiated to lower the potential side effects of cART and increase adherence¹⁷³. It offers two to three days off treatment every week, with the goal of maintaining virological suppression by optimizing adherence and improving quality of life. The BREATHER study¹⁷⁴ (BREaks in Adolescent and child THERapy using Efavirenz and two NRTIs) did not show, after 3.6 years, any

inferiority in patients treated with EFV based ART with two days a week off therapy compared to those with continuous treatment. Ninety percent of the participants reported that weekend break improved their quality of life. These findings need to be replicated with other ART regimens. Short cycle therapy (five days on and two days off) was implemented for 146 HIV-infected adults in Uganda for 72 weeks. Short cycle therapy was at least as effective as continuous therapy in terms of treatment failure¹⁷⁵.

Dual therapy in virologically suppressed patients is another potential way to reduce long-term toxicity, increase tolerability, reduce drug-drug interactions and improve adherence by decreasing the pill-burden. Different studies showed that DRV/r or ATV/r and 3TC dual therapies are not inferior to triple standard cART (ATV + 2 nucleos(t)ides)^{176,177}. Dual therapy was used in three RLS countries (Cameroon, Senegal and Burkina Faso) after viral suppression with boosted protease inhibitor plus NRTI in second-line therapy. Maintenance therapy with boosted protease inhibitor plus lamivudine was associated with a high rate of success at week 48 (97%) in 132 patients, despite the presence of M184V mutations at first-line treatment failure. LPV/r monotherapy was associated with 24.8% failure in 133 patients and was not recommended¹⁷⁸. Other RCTs on dual therapy are ongoing.

Finally, the use of long-acting injectable antiretrovirals was studied in two recent clinical trials (LATTE and LATTE-2). The combination of cabotegravir and rilpivirine administered every 4 or 8 weeks is well-tolerated and non-inferior to continuous oral therapy. The injectable treatment was generally well tolerated with only 2 of 230 patients (<1%) who had to discontinue due to significant injection-site reactions¹⁷⁹. A recent study using pharmacokinetic modelling simulated potential dosing strategies for existing long-acting injectable cabotegravir and rilpivirine in children and adolescents (aged 3–18 years)¹⁸⁰. Pain

related to administration of IM injections could possibly be a barrier for children who could prefer option for PO dosing regimens¹⁷⁹. In RLS, clean needle access and trained staff to administer injections will be additional logistical requirements. Capacity for injectable practices has been demonstrated in reproductive health (contraception) and in multi-drug-resistant tuberculosis (aminoglycosides)¹⁸¹. One more requirement is that the administering health center is readily accessible and does not require travel to a centralized high-level health center^{181,182}. Mobile health approaches could be provided by a traveling health group with the capacity to meet patients near to their residence¹⁸³⁻¹⁸⁶.

Generalize access to genotyping

Therapeutic education and assessment of adherence is the first unavoidable step to improve the care of HIV-infected patients under cART. Assessment of adherence may be difficult, and we demonstrated that 25 to 75% of the children with VF who have changed therapy had no HIVDR. In order to increase the chance to win this race against HIV by 2030, we advocate for the generalization of HIV genotyping before switching to a second line of treatment in RLS. This will make the present line of treatment more effective by avoiding unnecessary therapies switches in case of VF and guide the clinician for the choose of the second line of treatment. Moreover, national recommendations for cART initiation could be improved by assessing susceptibility to NRTI and NNRTI at baseline.

In our opinion, the latter recommendation will be difficult to implement for all infants or children and will only be performed as part of national HIVDR surveys unless genotyping prices go down very sharply.

Different initiatives have emerged to facilitate access to genotyping in RLS. The Southern African Treatment and Resistance Network (SATuRN) and the

Affordable Resistance Test for Africa (ART-A) group have developed laboratory methods to reduce the costs for resistance testing. Both used reduction in the number of primers, reduction of reagent volumes or simplification of PCR methods by targeting amplification of reverse transcriptase and protease fragments or reverse transcriptase alone¹⁸⁷⁻¹⁹⁰. In 2013, SATuRN collaborated with the Life technologies company to reduce the costs of PCR¹⁸⁷.

In RLS, only a small number of laboratories are equipped for genotyping. It follows that blood samples have to be transported to central laboratories. Transport of blood samples is facilitated by the use of dried blood spots (DBS). They are easy to collect, easily transported, and HIV-1 nucleic acids remain stable on DBS for long periods at ambient temperatures¹⁹¹. However, as our own experience has shown, the sensitivity is lower than from whole plasma specimens and depends largely on the HIV-1 VL¹⁹².

The reduction of genotyping cost and the possibility to transport blood samples in good conditions allowed SA to launch national resistance surveys. If these surveys are essential they will not be representative of all patients infected with HIV in the country. Implementation of resistance testing in the clinical care context would require substantial expansion of existing laboratory capacity with qualified personal but would improve the individual and collective quality of care⁹⁵.

SA is one of the most advanced countries in RLS for HIVDR follow-up. Most of the people have access to VL monitoring and selected patients (children with a history of unboosted protease inhibitor regimen, tuberculosis treatment concomitant with LPV/r regimen or second line cART failure) should have access to HIV sequencing. This is not the case in many other countries where VL monitoring is only beginning and genotyping is non-existent. In our opinion, many laboratories and medical staff will be trained and equipped for CD4⁺% and VL

monitoring in the next few years. We should not miss the opportunity to implement HIVDR monitoring in some of these teams at the same time. In countries where NNRTI are still widely used, resistance testing will be unavoidable to assess the effectiveness of the cART in the future and anticipate the risk of treatment failure. We also demonstrated that NRTI resistances are also present and could be a growing problem during the next years. Resistance testing may become necessary to assess the effectiveness of NRTI backbones in RLS. Genotyping should not be seen as a solution but as an important alarm signal for the problem of drug resistance.

Access to new drugs

Access to new drugs is crucial in case of extended resistances across the country. Some countries could decide to stop the use of NNRTI and use other drugs. The optimal time to introduce new antiretroviral drugs (more than 10% HIVDR against the molecule) could only be determined if extended HIV genotyping testing is performed in all the countries. It could be the time to make new drugs available in SA in regard of the high rate of NNRTI PDR in our ESC (24%). In children older than 3 years, US NIH recently recommended the use of PIs or INIs as initial therapy, in contrast to the WHO and PENTA guidelines which still recommend the use of PIs or NNRTIs^{8,21,29}. In SA, PIs are already used in children who initiated treatment before 3 years of age and our results show that they should continue with this regimen later in life if genotyping is not available. However, despite their good resistance profile, PIs induce higher serum lipids and have more drug interactions than INIs⁸.

Aware of the importance of INIs as part of the cART, the governments of SA and Kenya announced in 2017 a price agreement with the pharmaceutical firm Mylan and Aurobindo. A single-pill treatment containing DTG, TAF and 3TC should

progressively replace the present adult first-line regimens (EFV, TDF and FTC) in 2019. The costs should decrease from US\$ 110/year with the present regimen to US\$ 75 patient/year. There are still concerns about DTG, which possible increased risk of neural tube defects in infants born to Botswanian women who were initiated on DTG before conception. Four of 426 women receiving DTG gave birth to an infant with a neural tube defect, compared with 14 of 11 173 women receiving non-DTG-based regimens. Until more conclusive data is available, DTG based regimens are not recommended for women who do not have an effective, reliable method of contraception¹⁹³. Moreover, DTG is not well studied in TB patients and increases the risk of immune response inflammatory syndrome in individuals with low CD4 cell counts (IRIS)¹⁹⁴. Specific pediatric limitations have also to be mentioned. Contrary to RAL, DTG is not recommended under 30 kg of weight and the single-pill formulation is unfortunately not adapted for children. To our knowledge, there was no price agreement with pharmaceutical firms for the introduction of “child friendly formulations” for RAL or DTG in RLS, which limits their use and forces the local medical staffs to use PIs or NNRTIs for children with VF. Efforts will be necessary during the next years to include the pediatric population in this new era of treatment¹⁹⁵.

The rate of HIVDR against INIs is presently very low and switching from an NNRTI to an INI based regimen could then be considered in children.

Costs of genotyping is presently estimated around US\$ 85-112 per person (depending on the technique)¹⁹⁶. We could estimate a cost of US\$ 5.1 million to genotype all children with VF in SA (15% of 400.000). This cost could vary annually in function of the VF rate. If a price agreement of US\$ 75/person/year is found between pharmaceutical firms and SA (as in adults), all South African children could be treated for US\$ 30 million annually. In comparison, the annual costs to treat infants with LPV/r and older children with EFV-based regimens (with ABC

and 3TC) are respectively US\$ 291 to 598/person/year and US\$ 193 to 273/person/year depending of the age of the children and the formulation used¹⁹⁷.

INIs look to be a promising solution knowing their effectiveness and their low costs for treatment of HIV infected adults and children in RLS. However, if INIs may transitorily allow to avoid the use of genotyping at a clinical level, regular monitoring of HIVDR at a national level will be necessary to confirm the long-term effectiveness of INIs in RLS. Indeed, RAL has a relatively low barrier of resistance and could rapidly be inefficient in case of wide use in suboptimal conditions. Second generation INIs like DTG (and maybe in the future cabotegravir and bictegravir) is the first choice for adults due to his higher barrier of resistance¹⁹⁸. The P1093 study, aims to determine the safety and the tolerability of DTG in combination with other antiretrovirals, in infants, children and adolescents¹⁹⁹. Positive results could launch a novel treatment area with INIs at any age, but the completion date is estimated for the end of 2023.

V. Perspectives

I. Perspectives

The assessment of the feasibility and the relevance of our recommendations could be the proposed in future studies.

Implementation of genotyping in case of VF could be done at Edendale and other KZN HIV clinics in collaboration with a centralized HIV laboratory in Durban. We could define the precise conditions (definition of VF, questionnaires to assess adherence...) to send blood samples to the centralized laboratory and assess the effectiveness of genotyping to improve the care of HIV-infected children. The effectiveness of genotyping could be used at a clinical level to choose a second or a third line treatment and to improve adherence. Moreover, we could follow the spectrum of resistances to recommend the most effective first line therapy in newly infected children. Finally, we could follow the emergence of new mutations since 2014 in our cohorts (ESC and LSC) and highlight the risk factors for the emergence of these new HIVDR (present or previous treatment, adherence...).

Comparison of the effectiveness of PI vs INI or NNRTI vs INI with a NRTI backbone in pediatric patients could also be done. Tolerability and side effects of INIs in infants and children will have to be followed-up closely.

The effectiveness of one or many booster doses of HBV vaccine in HIV-infected children to increase the rate of protection against HBV infections should be studied. Implementation of regular checks of the immunity in annual blood samples could be interesting to assess the rate of HBV chronic infection at entry in adult care. This rate should normally decrease with a better immunization program.

Look for immunity against other preventable diseases included in the national program of vaccination could also be of great interest in this population.

Finally, instauration of a better patient census, with a computerization of the data could lead to a better follow-up. It is currently very difficult to find a patient in another clinic because the paper files are sorted by week and day of consultation at the clinic. It is also impossible to know if a patient has passed through the morgue without knowing the date of death even if you know his name, first name and birth date. The best way to implement data storage at a local and national level could be of great interest especially in the present study to reduce the number of patients lost to follow-up.

VI. Conclusion

I. Early treatment initiation: effectiveness of the international recommendations in RLS

Four years after initiation of this thesis, we are able to answer to our questions.

The first was: **“On the long term, is early treatment initiation at least as effective as late treatment initiation in terms of CD4⁺%, viral load and viral resistances for children living in a RLS of SA?”**

Our results in the ESC are compatible with at least even good immunological outcomes and virological outcome in terms of VL than in children treated later in life (LSC). Knowing the short-term benefits of early treatment initiation on infants' mortality, these results are reassuring.

However, it must be emphasized that children included in the ESC more often harbored NRTI and NNRTI proviral mutations.

The long-term persistence of NNRTI mutation is a risk factor of treatment failure in case of NNRTI initiation even after 3 years of age. Guidelines for cART initiation and switch therapies should be adapted in consequence in RLS.

Moreover, higher rates of NRTI mutations in the ESC could lead to a PI monotherapy, which is a risk factor to miss virological suppression.

The second was: **“What is the prevalence of HIV/HBV coinfection and what is the effectiveness of the national program of vaccination for children living in a RLS of SA?”**

We found low rates of HBV chronic infection in the two cohorts of HIV-infected and uninfected children. Vertical and horizontal transmissions before 5 years of age decreased in both cohorts since the introduction of the HBV vaccine¹⁴⁸⁻¹⁵².

However, low seroprevalence of anti-HBs was documented despite a high rate of vaccination declared by caregivers. Due to their weaker immune system, HIV-infected children had lower anti-HBs rates than the HIV-uninfected patients.

Without establishing regular monitoring of their immunity and adaptation of the vaccine schedule, prevalence of HBV could stay high in SA despite optimal vaccination coverage in HIV-infected patients. We would also suggest to check the HBV status in HIV infected children, which is not presently done routinely.

II. Effectiveness and efficiency: do we have to make choices?

We focused on the effectiveness of early cART initiation in RLS and showed the emergence of many NNRTI and NRTI resistances in our cohorts. Introduction of INIs could be a good alternative for NNRTI and maybe for PI if the price and the formulation are adapted for children living in RLS.

The WHO and the UNAIDS gave a very ambitious agenda with very short deadlines to overcome the HIV epidemic, but a choice could be necessary between the introduction of INIs and genotyping at a clinical level and with other health care measures, due to the lack of financial resources. Health authorities have to be efficient and could give up the option to genotype for the benefit of INIs that would give a more immediate positive effect.

In our opinion, genotyping should however be promoted at least at regional level for different reasons:

We do not know when INIs will be available at an affordable price for infants and children in RLS. Children will continue to use NNRTI with a significant risk of VF and should be monitored for HIVDR.

If INIs are available, only a small number of VF should occur after initiation of these molecules, which could restrict the number of genotyping required.

In case of VF under INIs, genotyping should certainly be done because it is a new line of treatment in RLS and HIVDR should promptly be described.

Genotyping should become more readily available with the ongoing efforts to reduce both the prize of blood transport and the genotyping itself.

Follow-up of NRTI HIVDR should also be continued to adapt the national recommendations for cART initiation in case of increasing resistances rates.

PMTCT decreased the global incidence of pediatric HIV infection from 490.000 in 2000 to 180.000 in 2017 and many efforts are being made to continue this decrease. In parallel, pediatric mortality dramatically decreased from 570.000 in 2005 to 120.000 in 2016. Children will become adults and will leave the cohort of infected children. Consequently, less and less children will need cART in the future, but they will need long-life treatment. Genotyping could be helpful to improve the care of this declining pediatric cohort. For all these reasons, we think that genotyping is an effective but probably also an efficient method to monitor the evolution of drug resistance in RLS and to adapt antiviral policy.

What we are working for

The world has launched a race against HIV and all patients, families, and medical staff have to run together if they want to win. Clear and personalized markers of treatment effectiveness have to be available to guide all these millions of runners on their roads. This thesis gives a picture of the outcome of HIV infected children treated in a real life setting of a RLS of SA. This work shows encouraging results but also challenges like HIV resistances and co-infections. During the next years, countries will still have to invest millions for the fight

against HIV. We hope that the present work and many others will highlight health deciders in their choices for the management of pediatric HIV in RLS.

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VIII. Publications

Effectiveness of the South African Expanded Program of Immunization Against Hepatitis B in Children Infected With Human Immunodeficiency Virus-1 Living in a Resource-Limited Setting of Kwazulu-Natal

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Prevalence of Human-Immunodeficiency-Virus/Hepatitis-B-virus (HIV/HBV) coinfection and HBV vaccination response in children are unknown in Kwazulu-Natal. This study included 183 HIV-infected and 108 HIV-uninfected children aged between 5 and 15 years screened for HBV infection and vaccination. HBV infection occurred in 2.1% and 0% of HIV-infected and uninfected children respectively. Serological response to immunization was shown in 15.8% and 61.1% of HIV-infected and uninfected children, respectively ($P < 0.001$). Even if prevalence of HBV infection was low in these cohorts, HIV-infected children will stay at risk of infection if the vaccine schedule is not adapted. **J. Med. Virol.** **89:182–185, 2017.** © 2016 Wiley Periodicals, Inc.

KEY WORDS: HIV/HBV co-infection; chronic hepatitis B; long-term outcome; resource-limited setting

[Healy et al., 2013]. Early initiation and better access to Highly Active AntiRetroviral Therapy (HAART) has allowed more children to reach adulthood, however chronic carriage of HBV constitutes a major risk factor of morbidity and mortality due to hepatic complications [Phung et al., 2014].

Since the implementation of hepatitis B vaccination in the Expanded Program of Immunization (EPI) in 1995, the apparent prevalence of chronic HBV infection decreased from 10.4% [Vardas et al., 1999] to 5–7% [Ott et al., 2012] in South African children. In 2001, evidence for complete elimination of HBs antigen (HBsAg) carriage has been reported in children under 5 years of age with unknown HIV status [Tsebe et al., 2001]. To our knowledge, since 2000, only two studies described the prevalence of chronic HBV (1.2–1.7%) in HIV-infected children living in South Africa (SA). Gauteng and North West provinces were mainly represented, with only a few patients who came from other provinces (Mpumalanga, Limpopo, and Northern Cape). [Simani et al., 2009; Amponsah-Dacosta et al., 2014].

INTRODUCTION

Among the estimated 35 million people affected by the Human Immunodeficiency Virus (HIV) worldwide [WHO, 2014], approximately 2–4 million are chronic carriers of Hepatitis B Virus (HBV) [Phung et al., 2014]. HIV/HBV coinfection worsens the natural progress of HBV infection because it decreases the chance to clear acute HBV infection, induces faster progression to cirrhosis and a higher risk of hepatocarcinoma

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Since its initiation in SA, hepatitis B vaccination coverage rates increased from 50% to 79% in the general population in 2011 [WHO/UNICEF, 2013]. However, HIV-infected children do not respond adequately to the routine immunization schedule due to a weaker immune system and no restoration of the HBV immunity after immune reconstitution [Healy et al., 2013]. Due to the absence of national HBV serosurvey in SA, data on the long-term impact of the vaccine on HIV-infected children are scarce.

Finally, HIV has a dramatic impact on families. Many children become orphans, are critically ill and their caregivers often ignore the patients' history. Record of vaccinations is often unreliable and not reflected by serological results.

This survey aims firstly to describe the prevalence of HIV/HBV co-infection in children living in a resource-limited setting (RLS) of the KwaZulu-Natal (KZN) province. Secondly, to compare the HBV vaccination response in HIV infected and uninfected children from this same region.

PATIENTS AND METHODS

Study Procedures

Children between 5 and 15 years of age were included on the basis of their HIV status just before their consultation with the local medical staff. Ten with uncertain HIV serological status were verified and were negative. Parents or caregivers of all subjects gave a written informed consent prior to any procedure. A questionnaire was filled with the investigator and blood samples were taken. Caregivers had to mention vaccination in infancy without indicating the number of doses administered. The study was approved by the South African ethical committee «Umgungundlovu Health District Review Board (UHERB)» and the Belgian Ethical committee «Comité d'Ethique Hospitalo-Facultaire Saint Luc—Université Catholique de Louvain».

Study Population and Setting

This survey was conducted in SA between September and December 2014 in the Edendale Regional Hospital located in a peri-urban area and RLS setting of the KZN province. The overall prevalence of HIV in the KZN province is the highest of SA with 16.9% of people infected [Shisana et al., 2014]. More than 3,000 patients (in majority mothers and their children) are currently on follow-up in a HIV family clinic built within the hospital complex.

One hundred and eighty-six HIV-infected children; 84 females (45.1%), with a median age of 9.1 years (range 6.5–14.8 years) were included in this study during their routine consultation at the HIV family clinic. Patients were distributed in two subgroups: the 5–10 years (103 children) and the 11–15 years (80 children). Three patients were excluded from the study due to technical failures during the laboratory analysis.

One hundred and seventeen HIV-uninfected children; 44 females (37.6%), with a median age of 9.0 years (range 5.8 and 15.0 years) were enrolled during their consultation at the emergency service of the hospital. Same distribution was done as in the other cohort: the 5–10 years (74 patients) and the 11–15 years (34 children) subgroups. Nine patients were excluded due to technical failures.

Laboratory Procedures

Blood samples were collected and sent to an external laboratory (Global Lab Durban, Amanzimtoti, South Africa). HBV serology including anti-HBs antibodies (anti-HBs), HBsAg and anti-HBc antibodies (anti-HBc) was performed by ADVIA Centaur XP immunoassay system (Siemens Healthcare GmbH, Henkestraat 127, 91052 Erlangen, Germany) with dedicated reagents following the manufacturer recommendations.

Statistical Analysis

Proportions between HIV infected and uninfected cohorts were compared using the Fisher exact *P*-value test to assess the significance of difference between the two cohorts. Chi-square trend test was used to study the significance of differences between the age subgroups in the two cohorts.

Definitions

- (i) Ongoing HBV infection: isolated positive HBsAg.
- (ii) Past HBV infection: positive anti-HBc with anti-HBs.
- (iii) Vaccinated against HBV: isolated detection of anti-HBs (≥ 10 mIU/ml)

RESULTS

Serological Markers of Past or Present HBV Infection

Non-significantly higher rates of HBV infection were found in the HIV-infected compared to the HIV-uninfected cohort ($P = 0.3$). Prevalence of HBV infection did not increase with age in both cohorts ($P = 0.8$ for HIV-infected and uncalculable for HIV-uninfected cohorts) (Table I).

Serological Evidence of Immunity

Prevalence of isolated anti-HBs (≥ 10 mIU/ml) was lower in HIV-infected than in HIV-uninfected children ($P < 0.001$). This was observed in both age subgroups (5–10 and 11–15 years) ($P < 0.001$). HIV-infected cohort had a significant lower immunity in the 11–15 years subgroup ($P = 0.035$) (Table II).

Evidence for Vaccination Against HBV According to the Road to Health Cards (Vaccination Card or RHC) and the Caregivers

In the HIV-infected cohort, 7/183 (3.8%) caregivers brought a RHC with proof of vaccination and 6/7

TABLE I. Serologic Markers of Past and/or Ongoing Infection in the HIV-Infected and Uninfected Cohorts

	HIV-infected			HIV-uninfected		
	5–10 years	11–15 years	Total	5–10 years	11–15 years	Total
Ongoing infection	0/103 (0%)	1/80 (1.3%)	1/183 (0.5%)	0/74 (0%)	0/34 (0%)	0/108 (0%)
Past infection	2/103 (1.9%)	1/80 (1.3%)	3/183 (1.6%)	0/74 (0%)	0/34 (0%)	0/108 (0%)

TABLE II. Comparison of the Immunity Against HBV in the HIV-Infected and Uninfected Cohorts According to the Age Subgroup of the Patients

	HIV-infected			HIV-uninfected		
	5–10 years	11–15 years	Total	5–10 years	11–15 years	Total
Presence of anti-HBs	21/103 (20.4%)	8/80 (10%)	29/183 (15.8%)	49/74 (66.2%)	17/34 (50%)	66/108 (61.1%)

(85.7%) had negative anti-HBs. In the HIV-uninfected cohort, 51/108 (47.2%) caregivers brought a RHC with proof of vaccination but 20/51 (39.2%) had negative anti-HBs despite records showing they had been fully vaccinated (three doses of vaccine).

Parents and caregivers of HIV-infected patients asserted that 138/183 (74.6%) of the children were vaccinated in infancy versus 99/108 (91.7%) in the HIV-uninfected cohort. Remaining caregivers did not remember if their child was vaccinated in infancy.

DISCUSSION

Despite a theoretical higher risk to develop chronic HBV, the 183 HIV-infected children included in this study had low rates of past and ongoing infections. No HIV-uninfected child had evidence of HBV carriage. Knowing that most of HBV infections are contracted before 5 years of age in Africa [Healy et al., 2013], these results are encouraging. A low rate of HBsAg (1.7%) was also found in 60 HIV-infected children between 1 and 16 years of age living in two other provinces of SA [Amponsah-Dacosta et al., 2014]. Previous studies in children younger than 5 years, showed ranges from 0.4% in children with unknown HIV status [Schoub et al., 2002] to 2.7% in HIV-infected children [Simani et al., 2009].

Prevalence of anti-HBs was significantly lower in HIV-infected (15.8%) versus uninfected children (61.1%). Immunity was not checked and no extra dose was administered after administering the three doses of vaccine at 6, 10, and 14 weeks in the HIV cohort. Standard immunization schedule was already shown to be suboptimal in immunosuppressed children [Lao-Araya et al., 2011; Sokal et al., 2013; Wilkins et al., 2013] and repeated or increased dose of vaccine after immune recovery may be considered [Wilkins et al., 2013]. Ideally, anti-HBs should be measured 4–8 weeks after the last vaccine dose and

one to three further doses should be administered in case of vaccine failure (Ab titer <10 mIU/ml). Immunocompromised subjects should be tested annually and revaccinated if anti-HBs <10 mIU/ml [Sokal et al., 2013; Spearman et al., 2013].

HIV-uninfected subjects included in the present study presented low serological rates (61.1%) of anti-HBs, even when they had a full vaccination schedule completed in their RHC. It tends to confirm waning of serological markers more than a lack of vaccination in this cohort. It is a well-documented phenomenon that does not necessarily imply a loss of protection against HBV [Liang et al., 2009]. HIV-uninfected children from other South African provinces had higher rates of anti-HBs (81.7%) with a clear pattern of waning immunity and rising HBV chronic carriage with increasing age. This was an argument to consider a hepatitis B vaccine booster dose for pre-adolescents [Amponsah-Dacosta et al., 2014]. We could not confirm rising of chronic HBV carriage with age in HIV-uninfected children due to the absence of infection in both age subgroups.

Parents or caregivers reported a high rate of vaccination, in both HIV-infected and uninfected children (74.6% and 91.7%, respectively) despite the low prevalence of anti-HBs in both groups. These reported results are compatible with a study which found 97%, 92%, and 74% vaccination rates at 6, 10, and 14 weeks in the province of KZN [Fadnes et al., 2011].

Because they were included in the study during routine consultation and as many caregivers were not the parents, only a few caregivers of HIV-infected children brought the RHC to the consultation.

This study has some limitations. It is a retrospective study limited to a single clinic with only 291 included children. Larger survey at regional or national levels would be of great interest. Vaccines' origin and composition were unknown and may be

different overtime with potential impact on immunogenicity. Finally, the site of injection and the storage methods of the vaccines were not specified, which could also influence the effectiveness of the vaccination [Weber et al., 1986].

In conclusion, this survey showed low rates of HBV chronic infection in HIV-infected and uninfected children living in a RLS of KZN province. Vertical and horizontal transmissions before 5 years of age decreased in both cohorts since the introduction of the HBV vaccine. However, low seroprevalence of anti-HBs was documented despite a high rate of vaccination declared by caregivers. Due to their weaker immune system, HIV-infected children had lower anti-HBs rates than the HIV-uninfected patients. Without establishing regular monitoring of their immunity and adaptation of the vaccine schedule, prevalence of HBV could stay high in SA despite optimal vaccination coverage in HIV-infected patients.

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Virological and Immunological Long-Term Outcome of Human Immunodeficiency Virus-1 Infected Children Treated before One Year and after Two Years of Age in a Resource-Limited Setting of South Africa

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Abstract

Introduction: Benefits of early Highly Active Antiretroviral Therapy (HAART) to reduce infant mortality and morbidity have been demonstrated in resource-limited and rich settings. However, immunovirological data collected in Sub-Saharan Africa are scarce. This study describes the long-term outcome of South African children who started HAART before one year of age (Early Starters Cohort or ESC) and compare their immunovirological outcomes to children who started their therapy after two years of age (Late Starters Cohort or LSC). Immunovirological results will be compared in order to evaluate the long-term non-inferiority of early treatment initiation.

Methods: Fifty-five children were included in the ESC (mean follow-up period 7.9 years) and 96 children were included in the LSC (mean follow-up period 6.3 years). Children from the ESC and the LSC were subdivided into three subgroups according to CD4% at HAART initiation (<15%, between 15-24% and ≥ 25%).

Results: All children included in the ESC achieved normal CD4% at least once during the entire follow-up period, contrary to the LSC where 89.6% children achieved normal CD4% ($p=0.014$). Furthermore, mean CD4% became higher in the ESC six years after treatment initiation. Children with CD4% between 15 and 24% at HAART initiation reached higher CD4% in the ESC, three years after treatment initiation. The proportion of children who experienced virological failure (>1000 cp/ml) was comparable in both cohorts but persistent undetectable viral load (<50 cp/ml) after initial virological suppression was more frequent in the ESC ($p=0.008$). Finally, the proportion of children with detectable viral loads (50 to 1000 cp/ml) at least once after initial virological suppression was higher in the LSC ($p=0.0022$).

Conclusion: HAART appeared highly effective in terms of immunovirological outcomes both in children treated before one and after two years of age. The results of this study demonstrate that early treated children more often achieved normal CD4%, tended to have higher mean CD4% and more sustained virological suppression. These results encourage the current international recommendations to initiate HAART as soon as possible in RLS.

Keywords: HIV; Children; Resource-limited setting; Long-term; South Africa; Treatment

Introduction

In 2010 the World Health Organization (WHO) recommended an immediate start of Highly Active Antiretroviral Therapy (HAART) in all patients diagnosed with HIV before 2 years of age [1]. This recommendation was extended to children younger than 5 years of age in 2013 [2] and to all children and adults in 2015 [3]. Benefits of early treatment initiation on infant mortality [4,5], neurodevelopmental outcome [5,6], growth recovery [7], immunologic restoration and virological suppression [8,9], have been demonstrated in short term follow-up studies implemented in resource-limited settings (RLS). A systematic review including 5 African studies, showed a 12 months HIV RNA suppression rate of 70% and a CD4⁺ cell percentage (CD4%) increase of 13.7%, for children initiating HAART in RLS [10].

Between 2005 and 2014, children treated with HAART increased from 71.500 to 824.000 [11] but long-term (>3 years) virological and immunological data collected in Sub-Saharan Africa are scarce [12-16]. Children are at risk to develop treatment failure due to inappropriate antiretroviral formulations, adherence difficulties, drug toxicity or development of resistance [17].

The aim of this study is to describe the long-term outcome in a

real life setting of children who started HAART before 1 year of age (early starters cohort or ESC) and after 2 years of age (late starters cohort or LSC) in the same RLS of KwaZulu-Natal, South Africa (SA). Immunovirological results will be compared in order to know if early treatment initiation, which is a major advantage in a short-term point of view, could lead to a poorer long-term evolution in terms of CD4% and viral load (VL).

Method

Study population and setting

This non-inferiority retrospective study was conducted in SA

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between September and December 2014 at Edendale Regional Hospital located in a peri-urban and RLS of KwaZulu-Natal. The overall prevalence of HIV is the highest of SA with 16.9% of people infected [18].

In total, hundred fifty-one children were included in the present study in the ESC and the LSC.

Children in the ESC were included in a previous study about feasibility and effectiveness of early HAART initiation in RLS, which took place between 2005 and 2008 [19]. First condition to be included in this previous study was to be younger than 1 year of age at HAART initiation (median 8.6 months, range 2.1–11.9 months). Other criteria to initiate HAART included positive HIV-1 DNA PCR, WHO stage 2-4 and/or CD4% <30% [19].

Children included in the LSC were invited to participate to the present study during a planned appointment in the HIV clinic. Inclusion criteria were to have initiated HAART after 2 years of age in the HIV clinic and to be vertically infected according to HIV status of the mothers. Age at HAART initiation had to be mentioned in the patient's file. These children were not included in the previous study between 2005 and 2008 because they were older than 1 year during this period or because they started HAART after 2008. They initiated HAART in function of their WHO clinical stage, CD4% or comorbidities following the successive South African guidelines between 2005 and 2012.

Children from both cohorts came from the same region with the same socio-economical environment. They were followed-up by the local staff between 2008 and 2014 and were treated following the national recommendations.

Study procedures

Parents or caregivers gave a written informed consent prior any procedure. Data were collected from the medical files (before 2010 records on paper and later on computer). The study was approved by the South African ethical committee «Umgungundlovu Health District Review Board (UHERB)» and the Belgian Ethical committee «Comité d'Ethique Hospitalo-Facultaire Saint Luc – UCL».

Definitions

Children with CD4% <15%, between 15-24% and ≥ 25% at treatment initiation were described as low (LIS), intermediate (IIS) and high (HIS) immunological subgroups.

Virological failure was defined as a VL above 1000 copies/mL based on the annual blood check.

Viral suppression was obtained when VL was less than 50 copies/mL.

Laboratory procedures

Blood samples were analyzed during routine consultation at the Edendale Hospital laboratory. CD4⁺ count was analyzed by flow cytometer FC 500 series[®] from Beckman Coulter (Paris France) and expressed as % of leucocytes and absolute value. VL was measured by real time PCR with the "COBAS[®] AmpliPrep/COBAS[®] TaqMan[®] HIV-1 Test, v2.0 (Roche Diagnostics)". The lower limit of quantification (LLOQ) was 20 copies/mL.

Current South African antiretroviral treatment guidelines

All infants and children under 3 years of age (or <10 kg) are treated with Abacavir (ABC), Lamivudine (3TC) and Lopinavir boosted

ritonavir (LPV/r) when they initiate their treatment. Children over 3 years of age are treated with ABC, 3TC and Efavirenz (EFV) [20]. Before 2010, guidelines recommended to start treatment with Stavudine (d4T) instead of ABC [21].

Statistical analysis

Immunological and virological mean values were compared with the Student t test. Proportions between the ESC and the LSC were compared with the exact Fisher test. Differences in CD4 means were also tested with the non-parametric Wilcoxon test, which resulted in p-values similar to the ones obtained with the t-test.

Results

Study population

From the 94 subjects described in the 2005-2008 study [19], 55 were included in the present research (23 females and 32 males). The remaining 36 patients were transferred to other centers and lost to follow-up (33/36) or died (3/36). Mean age at inclusion was 9.6 years (range 7.1-10.5 years) with a mean follow-up period of 7.9 years (range 6.8-9.8 years).

The control group (LSC) included 96 subjects (42 females and 54 males) who initiated HAART after 2 years of age (median 4.3 years range 2.0-7.6 years) between 2005 and 2012. Mean age was 10.6 years (range 6.6-14.8 years) and the mean follow-up period was 6.3 years (range 2.5-9.8 years).

Initial therapies

In the ESC, 98% of children were initiated on HAART with a regimen including one protease inhibitor (PI) and two nucleoside reverse transcriptase inhibitors (NRTIs) and 2% with one non-nucleoside reverse transcriptase inhibitor (NNRTI) and two NRTIs.

In the LSC, 12% of children were initiated on HAART with a regimen including one PI and two NRTIs (children between two and three years of age at treatment initiation) and 88% with one NNRTI and two NRTIs (children older than three years at treatment initiation) (Table 1). Only 5 children were followed-up after 8 years in each cohort; therefore the results are not shown for any longer period of time.

PI/NNRTIs	NRTIs	ESC	LSC
Lopinavir/ritonavir	Stavudine/Lamivudine	50/55 (91%)	12/96 (12%)
Lopinavir/ritonavir	Zidovudine/Lamivudine	4/55 (7%)	/
Nevirapine	Stavudine/Lamivudine	1/55 (2%)	/
Efavirenz	Stavudine/Lamivudine	/	55/96 (58%)
Efavirenz	Abacavir/Lamivudine	/	28/96 (29%)
Efavirenz	Zidovudine/Lamivudine	/	1/96 (1%)

PI: Protease Inhibitor; NNRTI: Non-Nucleoside Reverse Transcriptase Inhibitors; NRTI: Nucleoside Reverse Transcriptase Inhibitor; ESC: Early Starters Cohort; LSC: Late Starters Cohort

Table 1: HAART regimen at treatment initiation in early and late starters cohorts.

Switch of therapy

A large proportion of children have changed treatment in both cohorts mainly due to revisions of the national recommendations. ESC and LSC replaced d4T by another NRTI (ABC, AZT or TDF) in 78% and 52% of patients respectively regardless of virological failure.

Switch to a second line of HAART due to virological failure was not significantly different between the ESC (11%) and the LSC (14%) (p =

0.624). Successive lines of treatment after virological failure are detailed in Table 2.

Among the ESC, one patient replaced AZT for d4T due to anemia and one patient changed from LPV/r to EFV (reason not specified). Among the LSC, one child changed from EFV to LPV/r due to drowsiness (data not shown).

Immunological outcome

Mean CD4% at HAART initiation was not significantly different and increased in the same proportions, in the ESC and the LSC during the first five years after treatment initiation (+18.3% vs. +16.2%). The largest increase occurred during the first year of therapy in cohorts (+13.8% vs. +11.9%). Six years after treatment initiation, mean CD4% stayed significantly higher in the ESC (Figure 1 and Table 3).

All children included in the ESC (55/55) achieved normal CD4% at least once during the entire follow-up period, contrary to the LSC where 86/96 (89.6%) children achieved normal CD4% (p=0.014).

In a second analysis, results were subdivided in three subgroups in function of the CD4% at HAART initiation (as explained in the definitions paragraph). First subgroup is the LIS (47% of the ESC vs. 56% of the LSC), second is the IIS (37% of the ESC vs. 32% of the

LSC) and third is the HIS (16% of the ESC vs. 12% of the LSC). The proportion of children included in each subgroup was not significantly different in both cohorts (p=0.56, 0.47 and 0.74, respectively).

LIS from both cohorts had comparable mean CD4% at HAART initiation and during the entire follow-up period (p>0.05). Mean CD4% became higher than 25% in both cohorts after two years of treatment (Figure 2 and Table 4). In the ESC, 26/26 children achieved normal CD4% at least once during the entire follow-up period and 47/54 (87%) children achieved normal CD4% in the LSC (p=0.089).

IIS from both cohorts had comparable mean CD4% at HAART initiation but in children from the ESC it became and stayed significantly higher than in the LSC, three years after HAART initiation. Mean CD4% became higher than 25% in both cohorts after two year of treatment (Figure 2 and Table 4). In the ESC, 20/20 children achieved normal CD4% at least once during the entire follow-up period, and 27/30 (90%) children achieved normal CD4% in the LSC (p=0.27).

HIS from both cohorts had comparable mean CD4% at HAART initiation and during the entire follow-up period (p>0.05) except at six months of treatment (27.1% vs. 42.7%, respectively). Mean CD4% stayed higher than 25% in both cohorts during the entire follow-up period (Figure 2 and Table 4).

Number of patients ESC	First Line	Second Line	Third Line	Fourth line	Reason to change
1	AZT/3TC/NVP	AZT/3TC/LPV	ABC/3TC/EFV		//NVP+VF
1	d4T/3TC/LPV	ABC/3TC/EFV	AZT/3TC/LPV	TDF/3TC/LPV	//d4T+VF+anemia
1	d4T/3TC/LPV	d4T/3TC/EFV			VF
1	d4T/3TC/LPV	ABC/AZT/EFV			VF
1	d4T/3TC/LPV	ABC/3TC/EFV			VF
1	d4T/3TC/LPV	AZT/ABC/LPV/EFV			VF
Number of patients LSC	First Line	Second Line	Third Line	Fourth line	Reason to change
3	ABC/3TC/EFV	AZT/3TC/LPV			VF
1	ABC/3TC/EFV	ddl/AZT/LPV			VF
3	d4T/3TC/EFV	AZT/3TC/LPV			VF
1	d4T/3TC/EFV	ABC/AZT/LPV			VF
3	d4T/3TC/EFV	ddl/AZT/LPV	AZT/3TC/LPV		VF+ddl
2	d4T/3TC/EFV	ddl/AZT/LPV	ABC/AZT/LPV		VF+ddl
1	d4T/3TC/LPV	ddl/AZT/EFV	TDF/3TC/LPV		VF+ddl

//: No More Used Following National Recommendations; VF: Virological Failure; ESC: Early Starters Cohort; LSC: Late Starters Cohort; AZT: Zidovudine; 3TC: Lamivudine; NVP: Nevirapine; ABC: Abacavir; LPV: Lopinavir/Ritonavir; TDF: Tenofovir; d4T: Stavudine; ddl: Didanosine; EFV: Efavirenz

Table 2: Successive treatments after virological failure in the ESC and the LSC.

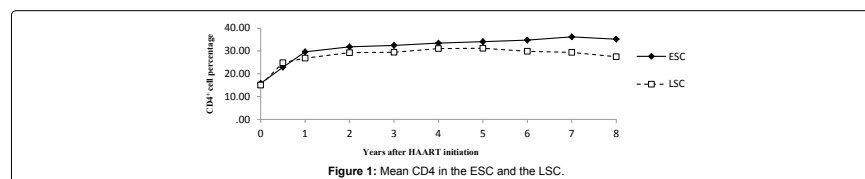


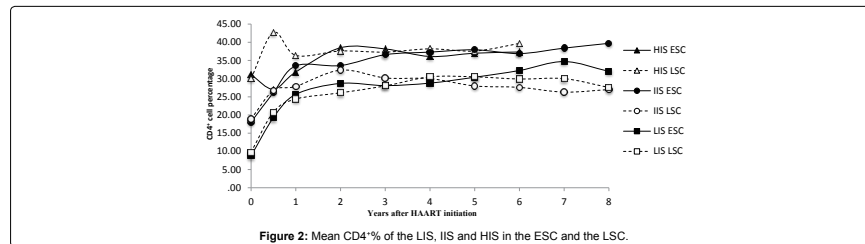
Figure 1: Mean CD4 in the ESC and the LSC.

	0 year	0.5 year	1 year	2 years	3 years	4 years	5 years	6 years	7 years	8 years
ESC mean CD4%	15.8 (55/55)	22.9 (52/55)	29.6 (47/55)	31.8 (47/55)	32.4 (47/55)	33.5 (49/55)	34.1 (47/55)	34.8 (52/55)	36.2 (33/55)	35.2 (17/55)
LSC mean CD4%	15.0 (96/96)	24.8 (75/96)	26.9 (72/96)	29.3 (77/96)	29.5 (79/96)	31.1 (66/96)	31.2 (51/96)	29.9 (51/96)	29.4 (32/96)	27.5 (17/96)
p value	0.59	0.26	0.088	0.13	0.064	0.13	0.067	0.042	<0.001	0.013

Numbers in brackets represent the number of data available for that year

Table 3: Mean CD4% in the ESC and the LSC during the entire follow-up period.

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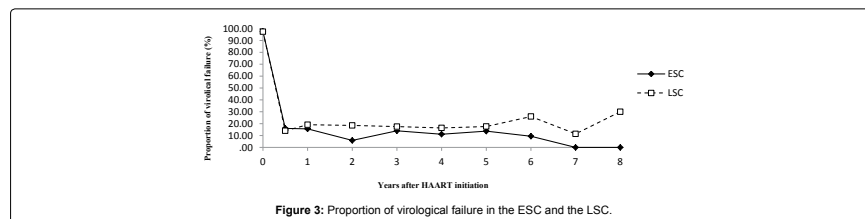
		0 year	0.5 year	1 year	2 years	3 years	4 years	5 years	6 years	7 years	8 years
LIS mean CD4%	ESC	8.8 (26/26)	19.3 (26/26)	25.7 (22/26)	28.7 (23/26)	28.1 (25/26)	28.8 (22/26)	30.4 (23/26)	32.2 (25/26)	34.7 (17/26)	32.0 (10/26)
	LSC	9.8 (54/54)	20.8 (43/54)	24.4 (41/54)	26.1 (44/54)	28.0 (46/54)	30.5 (38/54)	30.5 (32/54)	29.9 (35/54)	30.0 (23/54)	27.6 (14/54)
	p value	0.27	0.43	0.90	0.24	0.96	0.38	0.50	0.30	0.059	0.23
IIS mean CD4%	ESC	18.1 (20/20)	26.2 (18/20)	33.5 (19/20)	33.6 (18/20)	36.6 (17/20)	37.3 (18/20)	37.9 (18/20)	36.9 (18/20)	38.4 (13/20)	39.7 (7/20)
	LSC	18.9 (30/30)	26.8 (25/30)	27.9 (23/30)	32.3 (26/30)	30.2 (26/30)	30.0 (23/30)	28.0 (14/30)	27.6 (14/30)	26.3 (9/30)	27.0 (3/30)
	p value	0.245	0.847	0.038	0.636	0.031	0.008	0.0007	0.014	0.002	0.001
HIS mean CD4%	ESC	31.1 (9/9)	27.1 (8/9)	31.8 (6/9)	38.5 (6/9)	38.2 (6/9)	36.1 (8/9)	37.0 (8/9)	37.3 (9/9)	/	/
	LSC	30.1 (11/11)	42.7 (7/11)	36.4 (8/11)	37.6 (7/11)	37.3 (7/11)	38.2 (6/11)	37.6 (5/11)	39.7 (3/11)	/	/
	p value	0.63	<0.001	0.33	0.82	0.79	0.60	0.89	0.38	/	/

Numbers in brackets represent the number of data available for that year

Table 4: Variation of the CD4% in the LIS, the IIS and the HIS in function of the age at initiation.

		0 year	0.5 year	1 year	2 years	3 years	4 years	5 years	6 years	7 years	8 years
Viral loads log 10 (cp/mL)	ESC	6.5	4.2	3.4	2.9	4.8	3.9	3.5	4.6	1.6	1.6
	LSC	5.7	4.4	4.4	3.8	4.1	3.7	3.8	4.1	3.3	3.8
	p value	<0.001	0.72	0.21	0.047	0.31	0.79	0.41	0.39	0.054	0.11
Virological failure (%)	ESC	97.6	16.0	15.6	15.9	14.0	11.1	13.7	9.4	0.0	0.0
	LSC	97.4	14.0	19.1	18.5	17.5	16.4	17.6	26.0	11.4	30.0
	p value	1	0.085	0.66	0.066	0.63	0.43	1	0.038	0.078	0.014
Number of data/year	ESC	43	50	51	51	50	54	51	52	36	17
	LSC	77	89	89	83	80	65	51	50	28	15

Table 5: Viral loads and proportion of virological failure in the ESC and the LSC.



Virological outcome

Pre-HAART VL was significantly higher in the ESC but the difference in VL waned 6 months after treatment initiation (Table 5).

In the ESC, 17/55 (30.9%) children experienced virological failure at least once after treatment initiation, 25/55 (45.5%) never experienced virological failure and 13/55 (23.6%) had detectable viral loads between 50 and 1000 cp/ml at least one time during the entire follow-up period.

In the LSC, 36/96 (37.5%) children experienced virological failure at least once after treatment initiation, 19/96 (19.8%) never experienced virological failure and 41/96 (42.7%) had detectable viral loads between 50 and 1000 cp/ml at least one time during the entire follow-up period.

The proportion of children who experienced virological failure at least once after the first year of HAART was comparable in both cohorts when the entire follow-up period was considered ($p=0.48$). However, when every year of follow-up was analyzed separately, proportion of

virological failure was higher in the LSC ($p=0.038$ and 0.014) at six and eight years of HAART, respectively (Figure 3 and Table 5).

The proportion of children with persistent undetectable VL after initial virological suppression was higher in the ESC than in the LSC ($p=0.008$).

Finally, the proportion of children with detectable viral loads between 50 and 1000 cp/ml at least one time during the entire follow-up period was higher in the LSC ($p=0.0022$).

Discussion

In 2012, only 34% of the HIV-infected children were treated with HAART in the world [2]. The 2013 and 2015 WHO guidelines successively revised and simplified their recommendations to expand treatment in children and increase accessibility to HAART in RLS [2,3]. The present study evaluated the long-term impact and application of these recommendations in the field.

Both cohorts initiated treatment with three antiretroviral drugs as recommended by the WHO and the South African guidelines [2,20]. Children included in the ESC and the LSC initiated HAART with 2 NRTI and 1 PI or 2 NRTI and 1 NNRTI according to age, which means that the guidelines for the first line therapies are clear and correctly applied.

Second line therapies were more heterogeneous in both cohorts probably because different recommended options are available in the WHO [2] (e.g. LPV/r replaced by EFV, d4T+3TC replaced by ABC/TDF+3TC) and the South African guidelines [20] (e.g. LPV/r replaced by LPV/r+EFV or a 3rd line, d4T+3TC replaced by ABC+AZT). ddI is no more recommended in both guidelines but is still used in RLS. Finally, expert advice is recommended after first line failure with a LPV/r based treatment. Experts are not always available and the choice of treatment depends more often on the habits of the local staff.

Immunological outcomes were favorable for both cohorts (ESC and LSC) and for the three subgroups (LIS, IIS, HIS), which underlines the importance of initiating HAART regardless of age. Children included in the ESC had at least comparable (or even better) immunological outcomes than children included in the LSC supporting the non-inferiority of early treatment initiation, as recommended by the WHO and the South African guidelines.

Some favorable outcomes can be underscored. Before treatment, the ESC and the LSC both had a high proportion (47% vs. 56%) ($p=0.56$) of children with severe immunosuppression (<15% CD4%). This is consistent with a review from Koller et al. who reported 70% of severe immunosuppression in children <2 years of age who started treatment in low and middle-income countries in 2010 [22]. Surprisingly, patients included in the present study had comparable mean CD4% independently of age at HAART initiation. It was not the case in European and West African studies [14,23] who showed more severe immunosuppression when treatment was started later in life (-0.82% of mean CD4% per year of HAART initiation delay) [14]. This difference could be explained in our study by the exclusion of rapid progressors [24] who possibly died before 2 years (before HAART initiation) in the LSC.

Influence of age at baseline, independently of immunological status, was studied in European [23,25-29] and a few African cohorts (1-8.7 years follow-up) [14,30]. Results were inconclusive. Four studies [25-28] concluded the absence of influence of age at baseline and five studies [14,23,29-31] described poorer immunological outcomes for

late starters. The follow-up study of CHER [16] compared early time-limited HAART with deferred HAART in 377 South African infants with a median follow-up of 4.8 years. Early treated children (median age 7.4 weeks, CD4% of 35%) had better clinical and immunological outcomes than children treated later (median age 20 weeks, CD4% of 35%) even after planned treatment interruption at 40 or 70 weeks [16]. In our study, mean CD4% was normal in both cohorts. However, there were statistically less children who achieved normal CD4% in the LSC.

The European-American PENPACT-1 study [31], analyzed combined effects of CD4% and age at baseline. They found a significant interaction between CD4% and age on the probability of ever recovering a normal CD4% within 4 years. With increasing age, baseline CD4% had a stronger effect on the capacity to recover a normal CD4%. Children included in our three subgroups achieved normal mean CD4% independently of their immunological status at baseline. There was no statistical difference in the number of children who achieved normal CD4% in the LIS and the IIS, probably due to the small effectives in these subgroups.

In terms of virological outcome, VL was higher in the ESC at treatment initiation. This could be explained by their younger age and was already described in untreated perinatally infected infants, who had the highest HIV-1 RNA levels at 2 months of age followed by a slow decline during the first two years of life. These high VL and slow decline reflect the lower efficiency of the immature immune system, which cannot contain the viral replication and has possibly a greater number of HIV-susceptible cells [32].

The proportion of children, who experienced virological failure at least once after initial suppression, was not significantly different in both cohorts; with 30.9% and 37.5% in the ESC and LSC respectively. A recent retrospective study implemented in another rural area of South Africa found similar rates (38%) of virological failure (median duration since beginning HAART of 31 months) [13].

During the entire follow-up period, the proportion of children who sustained undetectable viral loads was significantly higher in the ESC and the proportion of children who experienced low-level viremia was higher in the LSC. Knowing that persistent low-level viremia is a risk factor of viral drug resistance [33,34], maintenance of an undetectable viral load decreases the risk to develop viral drug resistances in the ESC.

Limitations

This research has some limitations. It is a retrospective study that only includes a limited number of children who survived from 2005 until now and a number of children were lost to follow-up. Data collected at baseline could not be representative of all the infants from this region. Furthermore over the years some data were missing in the patients' files, which decrease the power of the study. However this is an operational study representing the real life on the field. Children included in the ESC and the LSC had, for most of them, different HAART regimens (PI vs NNRTI) at treatment initiation. A PI-based regimen initiation was described to be an advantage in terms of virological outcome in comparison with a Nevirapine-based regimen [35]. Our conclusions could be different if PI or NNRTI-based regimen were used in both cohorts.

Conclusion

HAART appeared highly effective in terms of immunological and virological long-term outcomes both in children treated before one year of age and after two years of age. The long-term results of this study

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demonstrate that early HAART initiation was certainly not inferior to late treatment. Children from the ESC, treated with a PI-based regimen, more often achieved normal CD4% and sustained virological suppression than children from the LSC. These results encourage the current international recommendations to initiate HAART as soon as possible in RLS.

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Authors Contributions

JCB and DVDL participated in the redaction of the questionnaires, interpretation of the results and redaction of the article. JCB, MK and LH recruited the patients in the HIV family clinic. JCB, PG and JR participated to the redaction of the questionnaires, interpretation of the results and redaction of the article. All authors have read and approved the final manuscript.

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Moving forward with treatment options for HIV-infected children

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ABSTRACT

Introduction: Current international guidelines recommend to treat all HIV-1 infected patients regardless of CD4 cell count. Despite the remarkable worldwide progress for universal access to antiretroviral during the last decade, the pediatric population remains fragile due to lack of randomized studies, inappropriate antiretroviral formulations, adherence difficulties, drug toxicity and development of resistance.

Areas covered: This review summarizes the latest recommendations and advances for the treatment of HIV-infected children and highlights the potential complications of a lifelong antiretroviral treatment initiated early in life.

Expert opinion: International guidelines recommend to start combination antiretroviral therapy (cART) as fast as possible in all children diagnosed with HIV-1. The principal goal is to improve survival and reduce mortality as well as rapidly decrease HIV reservoirs. This remains a challenge in resource-limited settings where diagnostic tools and treatment access may be limited. Different new strategies are in the pipeline such as immunotherapy in combination with very early cART initiation to seek remission or functional cure. For the time being and awaiting for long term remission or cure, there is a need for further pharmacokinetics studies, more pediatric formulations with improved palatability and implementation of randomized trials for the newer antiretroviral drugs.

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1. Introduction

In 2016, 36.7 million people, including 2.1 million children, were living with human immunodeficiency virus (HIV) infection [1]. Approximately 90% of these children are living in Africa [2]. Due to the increased access to combination antiretroviral therapy (cART) and early treatment initiation, pediatric mortality dramatically decreased from 570,000 in 2005 to 120,000 in 2016 [1,3].

Between 2000 and 2016, children receiving lifelong cART increased from 14,900 to 872,500, which represents 43% of the HIV-infected pediatric population [4]. In comparison, 53% of the HIV-infected adults received lifelong cART in 2016 [5]. However, great disparities persist between resource-rich settings like western countries where more than 95% of children have access to cART and resource-limited settings (RLS) like western, central, and North Africa or the Middle East where only 20% of children have access to cART [6].

Since 2010, conditions to start cART are less and less constraining. Between 2015 and 2017, international guidelines recommended immediate cART initiation in all HIV-infected children before immunosuppression and occurrence of symptoms [7–9]. Progressively, median CD4 cell counts at cART initiation are increasing worldwide, with very good results in some African regions (central Africa) [10].

Growing-up with HIV leads to many challenges for infected children and their caregivers. Management of adherence, resistance, side effects, and comorbidities is critical to the success of treatment [11].

Finally, the need for pharmacokinetic (PK) studies adapted to patient's ages and the relative paucity of pediatric formulations or dosage guidance are major challenges.

In this paper, we review the diagnosis and evidence for early treatment initiation in HIV-infected infants, the current recommendation for pediatric first- and second-line cART, new available drugs, as well as recent PK data concerning cART in children.

2. Early diagnosis of HIV infection in infants

2.1. Diagnostic methods

Early diagnosis of HIV infection in newborns has been recently reviewed [12]. We summarized here the most relevant information.

Before 18 months of age, HIV infection can only be definitely confirmed by the use of nucleic acid (DNA, RNA) testing technologies (NATs) [7,13,14]. Serological testing should be avoided before 18 months because maternal HIV antibody may be transmitted transplacentally and may persist in the child up to this age [7,15]. There are concerns about the

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Article highlights

- International guidelines recommend immediate cART initiation in all HIV-infected children.
- The FDA approved 23 of the 25 existing HIV antiretroviral drugs for pediatric use.
- Preferred treatment for HIV-infected children contains at least three drugs including either a PI, NNRTI or INI plus a dual NRTI backbone.
- Children are particularly at risk of poor adherence, which is the primary determinant of viral suppression, HIV transmission, disease progression and death.
- Adapted formulations, palatability and limited access to new drugs remain limiting factors to improve success of treatment in children.
- Long-term remission may be obtained with very early treatment initiation.

This box summarizes key points contained in the article.

possibility of false-negative results with NAT for infants infected at or around delivery. HIV may not be detectable by NAT for several days to weeks, due to cART taken by the mother or the infant for postnatal prophylaxis [16,17]. Despite low quality of evidence, available literature does not confirm that NAT performances at 4–6 weeks are lower in the context of cART exposure (pooled sensitivity and specificity of 100% and 99.0%) [7]. However, North American guidelines recommend to consider virologic diagnostic testing at birth and 2–4 weeks after cessation of antiretroviral (ARV) prophylaxis for HIV-exposed infants at high risk of perinatal HIV transmission. Virologic testing should also be performed for all HIV-exposed infants at age 14–21 days, at age 1 to 2 months, and at age 4–6 months. Confirmation of HIV infection should be based on two positive virologic tests from separate blood samples [14]. In RLS with low risks of transmission (<5% at 6 weeks), up to 70% of perinatal transmissions are expected to occur intrauterine and intrapartum. Adding birth testing may therefore be considered, with a retesting of positive results by NAT. Antiretroviral therapy (ART) should be initiated before the results of the second test are available because of the high risk of mortality with *in utero* infection. If the second test is negative, a third NAT should be done and also be negative before interrupting cART. A large number of infants will need to be tested to identify one infected infant (about 27:1). Lack of resources may therefore be a limitation [7]. In settings where transmission risk is high (>5% at 6 weeks), the proportion of children with *in utero* infection is lower, but the overall number of HIV-infected children will be higher. Birth testing can therefore have a high yield at the first test (about 4:1). However, the negative predictive value of the test at birth will be lower. It is critical to ensure retention in the testing cascade and to actively track infants who test NAT negative at birth [7]. Despite international recommendations, access to early infant diagnosis was available for only 50% of all HIV-exposed infants before 2 months of age in 2014, and it is estimated that currently only 30% of perinatally infected infants will start cART in a timely manner [7].

The use of rapid diagnostic test (RDT) and p24 antigen may be helpful to detect exposure to HIV in RLS [7]. They are not recommended for infant diagnosis in the North American guidelines because the sensitivity and specificity are lower than NAT [18,19].

After 18 months of age, HIV infection can be diagnosed with serological testing. Studies have shown that available RDT had an average sensitivity of 97.6% and average specificity of 99.1% [20]. Fourth-generation laboratory tests have a sensitivity and a specificity of >99% [21]. However, North American and European guidelines recommend performing NAT in children aged between 18 and 24 months with positive or equivocal serological testing because some cases of residual maternal HIV antibodies have been described [22,23].

2.2. Importance of early diagnosis and treatment

Up to 52% of children born with HIV in RLS will die before the age of 2 years in the absence of any ARV treatment [24,25]. By 5 years of age, the risk of mortality and disease progression in the absence of treatment falls to rates similar to those of young adults [26,27].

Based on new data obtained in RLS, the World Health Organization (WHO) recommended in 2010 an immediate cART initiation for children younger than 2 years. The Children with HIV Early Antiretroviral Therapy (CHER) study compared the outcome of HIV-infected infants (6–12 weeks of age) who received deferred ART on the basis of clinical or immunological criteria with infants who immediately initiated cART after diagnosis. Immediate ART reduced early infant mortality by 76% and HIV progression by 75% [28]. During the following years, the WHO recommended to start cART in all children younger than 5 years [29] and finally in all children independently of their age, clinical, immunological, or virological status [7]. This latest recommendation is based on the Strategic Timing of Antiretroviral Therapy (START) and the Trial of Early Antiretrovirals and Isoniazid Preventive Therapy in Africa (TEMPRANO) studies [30,31], which demonstrated benefits of cART initiation in asymptomatic adults regardless of CD4 cell count. Early cART initiation allowed control of viral replication before HIV evolved into more pathogenic quasispecies, induced fewer drug resistance mutations at virologic failure in adults [32], slowed immune system destruction and preserved immune function [33].

3. Current treatment guidelines for children

Currently, 25 HIV ARV drugs and 2 PK enhancers have received approval by the Food and Drug Administration (FDA) for use in HIV-infected adults and adolescents; the enhancers are used to increase the effectiveness of some ARVs [34] (Table 1). Remarkably, 23 of them are presently approved by the FDA for pediatric use [14] compared to 17 in 2009 [35] (Table 1). Only 15 of these 23 ARV drugs have adapted formulations (oral solutions, oral powder, chewable tablets, or injections) for children. An exhaustive list of the ARV drugs – including pediatric dosing, toxicity, and drug–drug interactions – can be found in the NIH guidelines [14]. European regulations are available on the European Medicine Agency's site (<http://www.ema.europa.eu/ema/>).

According to international guidelines developed by the WHO [7], the Paediatric European Network for Treatment of AIDS (PENTA) [8], and the North American guidelines [14], the preferred treatment for HIV-infected children is a combination

Table 1. HIV antiretroviral drugs currently approved by the FDA [12].

Class	Drug	Abbreviation	FDA approval
NRTIs/NRTIs	Abacavir	ABC	≥3 months
	Didanosine	ddl	≥2 weeks
	Emtricitabine	FTC	From birth
	Lamivudine	3TC	From birth
	Stavudine	d4T	From birth
	Tenofovir disoproxil fumarate	TDF	≥2 years
	Tenofovir alafenamide fumarate	TAF	≥12 years + ≥35 kg
	Zidovudine	AZT	From birth
	Efavirenz	EFV	≥3 months + ≥3 kg
	Etravirine	ETR	≥6 years
NNRTIs	Nevirapine	NVP	≥1 month ^a
	Rilpivirine	RPV	≥12 years + ≥35 kg
	Atazanavir	ATV	≥3 months + ≥5 kg
PIs	Darunavir	DRV	≥3 years + ≥10 kg
	Fosamprenavir	FPV	≥6 months
	Indinavir	IDV	Not for children
	Lopinavir	LPV	≥2 weeks
	Nelfinavir	NFV	≥2 years
	Saquinavir	SQV	Not for children
	Tipranavir	TPV	≥2 years
	Enfuvirtide	T20	≥6 years
	Maraviroc	MVC	≥2 years + ≥10 kg
	Dolutegravir	DTG	≥30 kg
INIs	Eltigravir	EVG	≥35 kg
	Raltegravir	RAL	≥1 month + ≥3 kg ^a

NRTIs/NNRTIs: nucleoside and nucleotide analog reverse-transcriptase inhibitors; NNRTIs: non-nucleoside analog reverse-transcriptase inhibitors; PIs: protease inhibitors; INIs: integrase inhibitors.

^aInvestigational doses for neonates <1 month of age exists but are not approved by the FDA.

Table 2. Preferred first-line regimens recommended by the WHO, US NIH and PENTA guidelines.

	WHO [6]	USA [12]	Europe [7]
≥12 years	TDF + 3TC/FTC + EFV	2 NRTIs ^a + ATV/r or DTG or DRV/r or EVG/cobicistat	TDF + FTC + ATV/r or DRV/r or EFV
6–12 years	ABC + 3TC + EFV	2 NRTIs ^a + ATV/r or DTG	ABC + 3TC + EFV or ATV/r ^b
3–6 years	ABC + 3TC + EFV	2 NRTIs ^a + ATV/r or DRV/r or RAL	ABC + 3TC + EFV or LPV/r ^c
≤3 years	ABC/AZT + 3TC + LPV/r	2 NRTIs ^a + LPV/r or RAL ^d or NVP ^e	ABC + 3TC + LPV/r or ABC + AZT + 3TC + NVP ^f

^a<12 years: ABC or AZT + 3TC or FTC; ≥12 years: ABC or TAF + 3TC or FTC (TAF always associated with FTC).

^bBetween 6 and 12 years of age.

^cBetween 3 and 6 years of age.

^dIf >2 years old.

^eIf <2 weeks of age.

^fIn children <3 years, add AZT to NVP-based regimens if high viral load (VL) or central nervous system (CNS) involvement until VL has been suppressed for at least 3 months. Four-drug induction for infants on NVP-based therapy may be considered until VL has been suppressed for at least 3 months, followed by three-drug maintenance therapy.

with at least three drugs including either a protease boosted inhibitor (PI), non-nucleoside reverse-transcriptase inhibitor (NNRTI), or integrase inhibitor (INI) plus a dual nucleoside analog reverse-transcriptase inhibitor (NRTI) backbone (Table 2).

Despite the lack of direct comparison in children, the recommended NRTI backbones are abacavir (ABC) + lamivudine (3TC) between 3 months and 12 years of age and ABC + 3TC or tenofovir disoproxil fumarate (TDF)/tenofovir alafenamide (TAF) + emtricitabine (FTC) for >12 years of age (not sexually mature) [7,14]. US guidelines (US NIH) recommend zidovudine (AZT) + 3TC before 3 months of age. FTC may replace 3TC at any age.

In children younger than 3 years, ritonavir-boosted lopinavir (LPV/r)-based regimen is preferred to nevirapine (NVP)-based regimen from 2 weeks of life. It confers reduced risk of discontinuing treatment, virological failure due to NNRTI resistance [36,37], and mortality regardless of previous NNRTI

exposure [11,38]. There are currently no data suggesting that initiating treatment within the first 2 weeks of life improves children's outcome [14]. However, US NIH recommends starting treatment with AZT + 3TC + NVP if initiated before 2 weeks of age.

In children older than 3 years, US NIH recently recommended the use of PIs or INIs as initial therapy, contrary to the WHO and PENTA guidelines which still recommend the use of PIs or NNRTIs [7,8,14]. In RLS, efavirenz (EFV) remains the only widely accessible option for children older than 3 years, and it ensures harmonization of regimens across age groups [7].

3.1. Nucleoside and nucleotide analog reverse-transcriptase inhibitors

NRTIs (Table 1) were the first class of ARV drugs available for the treatment of HIV infection. NRTIs are nucleoside analogs

that act as competitive inhibitors of HIV-1 reverse-transcriptase enzyme responsible for the reverse transcription of viral RNA into DNA. Incorporation of NRTIs into a growing DNA chain results in its termination.

Since 2011, TDF is approved for use in children as of the age of 2 years, providing the opportunity to offer the same regimen (and consequently to improve access to treatment) to both adults and children [7]. Other benefits of TDF include the ability to combine it with 3TC or FTC and EFV to create a potent single tablet regimen (STR) for children [39,40]. However, experience with TDF in young children is limited. TDF has been associated with reduced bone mineral density and impaired renal function [41,42]. In the UK and Ireland, 7.5% of children experienced severe side effects; half were renal toxicity [43]. Finally, TDF formulations for younger children are not widely available, and there is no study that directly compares TDF with AZT or ABC [7]. TDF + FTC or TDF + 3TC is the preferred NRTI backbone for children ≥ 2 years of age coinfecting with HIV and HBV and can be used for children coinfecting with tuberculosis (TB) [7,14].

TAF is an oral prodrug of TDF recently approved by the FDA for the treatment of HIV infection in ARV-naïve individuals aged ≥ 12 years with a creatinine clearance ≥ 30 mL/min. Compared to TDF, TAF induces fewer renal and bone adverse effects due to a higher stability in the plasma, resulting in a higher intracellular and a lower plasma TDF concentration [14]. However, TAF presents some other side effects, especially increased serum lipids. TAF plus FTC has been added in the last US NIH guidelines as a preferred dual-NRTI combination for adolescents aged > 12 years.

ABC is available in pediatric formulation and has a favorable resistance profile like TDF. In infants initiating cART with liquid formulations, twice-daily dosing is recommended with consideration of a switch to once-daily dosing 6 months after treatment initiation when viral load is undetectable and CD4 cell count is stable [7]. Furthermore, ABC is generally well tolerated, without the risks of bone and renal toxicity [7]. However, it is significantly more costly [7] than other NRTIs, and fatal hypersensitivity cases have been described among people with HLA-B*5701 allele (3% to 4% of Caucasian and Asian children, but very rare among African children [$< 1\%$]) [44].

One randomized controlled trial conducted in a large cohort of African children (1 month to 13 years of age) demonstrated that ABC and AZT were comparable in their clinical, immunological, and virological response, as well as safety and tolerability [45]. However, the choice of first-line NRTIs affects second-line ART, and failure of AZT results in the accumulation of thymidine analog mutations, reducing susceptibility to ABC or TDF in a subsequent regimen. For these reasons, ABC + 3TC should remain the preferred option for the first-line NRTI backbone in children in this age group. By contrast, the most frequent resistance mutations to ABC preserve and some even increase the susceptibility of HIV to AZT for second-line use [46]. Several studies suggest increased risk of myocardial infarction in adults with recent or current use of ABC [47,48]; however, other studies have not substantiated this finding [49,50], and there are no data in children [14].

Since 2010, stavudine (d4T) is no longer recommended by the WHO because of its known long-term toxicity (Table 3),

and AZT is not recommended in regions with risk of anemia such as malaria-endemic settings [7].

3.2. Non-nucleoside analog reverse-transcriptase inhibitors

NNRTIs (Table 1) interfere with reverse transcriptase by directly binding to the enzyme. They are not competitive with NRTI as they work at a different site on the reverse-transcriptase enzyme and are not incorporated into the viral DNA. NNRTIs have long half-life, which allows less frequent administration. They have a low risk of dyslipidemia (particularly NVP) and fat maldistribution and a lower pill burden than PIs. On the other hand, a single viral mutation can confer a high-level drug resistance (except etravirine [ETR]), and cross-resistance to other NNRTIs is common [14].

Table 3. Selected adverse events (non-exhaustive list) of antiretroviral medicines [12].

NRTIs	ABC	Hypersensitivity in HLA B*5701
	ddl	Neuropathy, lactic acidosis, pancreatitis, portal hypertension, diabetes
	FTC	Hepatitis after FTC discontinuation if hepatitis B virus coinfection
	3TC	Hepatitis after 3TC discontinuation if hepatitis B virus coinfection
	d4T	Peripheral neuropathy, lipodystrophy, pancreatitis, lactic acidosis, diabetes
	TDF	Asthenia, proximal renal tubular dysfunction, decreased bone mineral density
	TAF	Asthenia, headache, diarrhea, nausea, increased lipidemia
	AZT	Anemia, neutropenia, lactic acidosis, diabetes, lipodystrophy, myopathy
	NNRTIs	Rash, insomnia, psychosis, seizures, suicidality, hepatitis, lipohypertrophy
	EFV	Nausea, Stevens-Johnson syndrome, hypersensitivity reactions (hepatic failure), diarrhea
PIs	ETR	Rash (Stevens-Johnson syndrome), hepatitis, hypersensitivity syndrome (shock)
	RPV	Depression, insomnia, headache, Rash (DRESS), hepatotoxicity
	ATV	Hyperbilirubinemia, PR prolongation, nephrolithiasis, hepatitis
	DRV	Rash (Stevens-Johnson syndrome), hepatotoxicity, increased transaminases
	FPV	Rash (Stevens-Johnson syndrome), headache, nephrolithiasis, hepatitis
	IDV	Nephrolithiasis, hyperbilirubinemia, blurred vision, thrombocytopenia, anemia
	LPV	Hepatitis, PR/QT prolongation, cardiotoxicity increased in premature infants
	NFV	Hepatitis, diarrhea
	SQV	Headache, hepatitis, PR and QT prolongation
	TPV	Intracranial hemorrhage, rash, hepatitis
Entry inhibitors	T20	Injection-site reactions (98% patients), pneumonia, hypersensitivity reaction
	MVC	Cough, dizziness, musculoskeletal symptoms, fever, hepatitis, hypotension
	INIs	Insomnia, headache, hypersensitivity (rash, organ dysfunction)
INIs	DTG	Diarrhea
	RAL	Stevens-Johnson syndrome, fatigue, insomnia, fever, muscle weakness, rhabdomyolysis

For all PIs: fat maldistribution, hyperlipidemia and hyperglycemia, gastrointestinal intolerance (nausea and diarrhea), and possible increase in bleeding episodes in patients with hemophilia.

NNRTIs: nucleoside reverse-transcriptase inhibitors; NNRTIs: non-nucleoside reverse-transcriptase inhibitors; PIs: protease inhibitors; INIs: integrase inhibitors; DRESS: Drug Rash with Eosinophilia and Systemic Symptoms.

EFV-based regimens are virologically superior to regimens containing LPV/r [51] or non-inferior to raltegravir (RAL) [52], atazanavir (ATV) [53], maraviroc (MVC) [54], NVP [55], rilpivirine (RPV) [56], and elvitegravir (EVG) [57]. EFV has a better short-term toxicity profile than NVP [58]. However, children who are well controlled and stable on NVP-containing regimens do not need to substitute EFV for NVP [7]. One trial showed that dolutegravir (DTG) in combination with 3TC and ABC was superior to EFV with TDF and FTC after 144 weeks of follow-up. However, the differences were most likely due to drug discontinuation in the EFV group [59]. Adverse side effects associated with the use of EFV, notably its CNS side effects (neuropsychiatric toxicity in particular), remain a major concern. In one case series, 24% of children had at least one EFV-related CNS manifestation with 18% requiring drug discontinuation [60]. US NIH guidelines recently classified EFV plus two NRTIs as alternative rather than preferred regimen for children ≥ 3 years to <12 years of age.

ETR has a better CNS safety profile than EFV and has a higher resistance genetic barrier than other NNRTI(s) [14]; however, it is associated with multiple interactions with different boosted and unboosted PIs and must be administered twice daily. ETR is currently approved for ARV-experienced children [14]. The FDA approved its use for children ≥ 6 years [61], and studies in infants and children aged 2 months to 6 years are under way [14].

The Evidence for Contraceptive Options and HIV Outcomes (Echo) and the rilpivirine (RPV) versus efavirenz with two background nucleoside or nucleotide reverse-transcriptase inhibitors in treatment-naïve adults infected with HIV-1 (Thrive) study groups demonstrated non-inferior efficacy and more favorable tolerability of RPV versus EFV in treatment-naïve, HIV-1-infected adults. Patients ($N = 1368$) received RPV or EFV once daily, with two background nucleoside/nucleotide reverse-transcriptase inhibitors. RPV and EFV had comparable responses at week 96. RPV had more virologic failures but improved tolerability versus EFV with significantly fewer nervous system and psychiatric adverse events ($p = 0.001$) and significantly fewer discontinuations due to adverse events (3% versus 11%, respectively). The majority of virologic failures occurred in the first 48 weeks. However, RPV showed higher rates of virological failure in subjects with baseline HIV viral loads $\geq 100,000$ copies/mL. For this reason, initial therapy with RPV is only licensed in patients older than 12 years with HIV viral loads $\leq 100,000$ copies/mL [56,62–64].

3.3. Protease inhibitors

PIs (Table 1) block the activity of the protease enzyme, used by the HIV to break up large viral polyproteins into the smaller pieces required for viral replication and assembly of new viral particles. While HIV proviral genomes can still be expressed in the presence of protease inhibitors, the resulting virions are unable to infect new cells. Because it is recommended to use ritonavir as a PK enhancer, it is not included in this review as a protease inhibitor [14].

LPV/r has been studied in both ARV-naïve and ARV-experienced children and has demonstrated durable virologic activity and low toxicity [65–67]. It is known to have a good

resistance profile, which protects against the selection of NRTI resistance without compromising the use of other PIs in second-line regimens [68,69]. LPV/r syrup requires a cold chain and has a bad taste with the potential risk for suboptimal adherence. Heat-stable pellets, which have a better taste, are more acceptable to parents and caregivers [70], but there is still some uncertainty regarding the most appropriate way to administer the pellets to less than 3-month-old breastfed infants [71]. In settings where LPV/r syrup is not available and LPV/r pellets are the only formulation available, NVP should be continued until the age of 3 months [7]. If initiating cART in an infant less than 2 weeks of age, a regimen of AZT + 3TC + NVP should be started and NVP substituted with LPV/r at the earliest opportunity, preferably at 2 weeks of age, when LPV/r syrup can be used [7].

Substitution of LPV/r with an NNRTI after viral suppression is safe and efficient [72,73]. This strategy aims to reduce exposure to LPV/r, offer an easier approach to maintaining treatment, and preserve PI-based therapy for second-line ART. However, this approach adds complexity to treatment programs and requires access to virological monitoring [7]. Dosages of LPV/r for children younger than 6 weeks should be calculated based on body surface area. Due to higher clearance, the dose must be higher in infants aged <6 months (LPV 300 mg/m² twice daily) than in older children (LPV 230 mg/m² twice daily) [68]. In infants aged <6 weeks, it is recommended to adjust the dosage for incremental growth at more frequent intervals in the first months of life [74]. Finally, administering LPV/r with TB treatment is complex because drug levels are reduced by rifampicin [7].

Ritonavir-boosted atazanavir (ATV/r) is a well-tolerated and efficient alternative to LPV/r for children ≥ 3 months and ≥ 10 kg [75]. It induces less frequent gastrointestinal side effects and has a more favorable lipid profile. The drug has a once-daily dosing and a low cost. However, a usually mild and transient hyperbilirubinemia is found in 50% of cases [76].

Ritonavir-boosted darunavir (DRV/r) has a high genetic barrier to resistance and good safety profile [77]. However, it is not approved for children younger than 3 years, there is no suitable co-formulation with RTV, and it is significantly more expensive [7]. DRV/r was well tolerated and efficient as initial therapy in adolescents aged 12–18 years [78]. As replacement therapy, DRV/r was used successfully twice daily in children between 3 and 17 years [77]. Darunavir (DRV) boosted with cobicistat is approved in adults only [14].

Unboosted PIs such as fosamprenavir (FPV), DRV, and ATV and some boosted PIs such as indinavir (IDV), saquinavir (SQV), FPV, and tipranavir (TPV) are associated with reduced viral suppression, high pill burden, and higher frequency of side effects. For these reasons, their use is discouraged [7].

3.4. Entry inhibitors

Entry inhibitors (Table 1) block the entry of HIV into the cell by preventing the HIV envelope from merging with the host CD4 cell membrane. Two drugs are available for children. Enfuvirtide (T20) is currently approved in ARV-experienced children older than 6 years, but it is not commonly used

because of its high cost, need for twice-daily subcutaneous injections, and high rate of injection-site reactions [14]. Based on PK studies [79], the recommended dose for children aged 6–16 years is 2 mg/kg (maximum 90 mg) twice daily. Further data are needed for dosing in children aged <6 years [7]. T20 is no longer prescribed in adults.

MVC is an oral agent that alters the structure of the CCR5 chemokine receptor, preventing HIV to use it as co-receptor. The FDA recently approved its use for children ≥ 2 years + ≥ 10 kg [14,80]. The drug has multiple drug interactions and must be administered twice daily. In addition, tropism assays must be performed prior to use to ensure the presence of CCR5-tropic virus only [14].

3.5. Integrase inhibitors

These drugs (Table 1) block the viral integrase, preventing the step of DNA strand transfer of viral genome into the host cell genome.

RAL is effective at controlling viral replication and improving CD4 cell counts in treatment-experienced children infected with multidrug-resistant HIV and has been studied in children as young as 4 weeks of age [81]. RAL is thus the only integrase inhibitor to be approved in very young children. It could be considered as a first-line ARV drug [82] in case of poor tolerability or administering difficulties with LPV/r or in settings with very high risk of NNRTI resistance [7]. The existing RAL granule formulation requires reconstitution in water, which may be a risk factor of infection in RLS. Chewable tablets could be an alternative for infants, but data are not yet available [7]. RAL has a low potential for drug–drug interactions, though the dose of RAL must be doubled when administered with rifampin [83]. It is well tolerated in children, though the development of mutations associated with RAL resistance is a concern.

EVG has been approved by the FDA for the treatment of HIV infection in cART-naïve or cART-experienced patients older than 18 years of age. It is available in a fixed-dose single-daily tablet co-formulated with FTC, TDF, or TAF and boosted by cobicistat, as well as in a stand-alone formulation for coadministration with ritonavir or cobicistat. There are currently several EVG research studies in children and adolescents. The initial results of the pharmacokinetics, safety, and efficacy of the EVG/FTC/TDF/cobicistat STR in treatment-naïve adolescents [84] or combined with PI in virologically suppressed or failing HIV-infected children (6–12 years of age) [85] are encouraging. Clinical trials with EVG co-formulated associated with cobicistat, 3TC, and TDF [86] or TAF [87] are ongoing.

DTG is safe and well tolerated with a high barrier to resistance and maintains activity in the face of many mutations conferring RAL or EVG resistance. DTG is also available in fixed-dose combination tablets associated with ABC and 3TC [14]. One trial implemented in two age groups of 12–17-year and 6–12-year-old children showed favorable safety profile, virologic efficacy, and improving in CD4 number and percentage [88,89]. Because of its virologic efficacy, lack of drug interaction, and favorable toxicity profile, DTG (plus two NRTIs) has

been added in the last US NIH guidelines as a preferred initial regimen for children aged ≥ 6 to <12 years (weighing ≥ 30 kg).

3.6. PK enhancers

Cobicistat is used in adolescents and adults as a PK enhancer (boosting agent) of two protease inhibitors (ATV and DRV) and one integrase inhibitor EVG. The FDA does not approve it for children <12 years of age in combination with EVG, FTC, and TAF [14]. Ritonavir is used as protease enhancer with PIs [14].

4. Treatment failure

Of the 1.5 million children estimated to be in need of cART by 2020, up to 20% are expected to experience virological failure [90]. Effective first-line regimen and optimal adherence are critical to ensure durability and effectiveness of cART [91].

4.1. Adherence

Adherence to cART is the primary determinant of viral suppression, HIV transmission, disease progression, and death [92,93].

Children are particularly at risk of poor adherence because they are dependent on a responsible caregiver, who may also be infected with HIV. Suboptimal HIV care for family members could result in suboptimal care for the child [7]. Limited choice of pediatric formulations, poor palatability, large pill size or high pill burden, and unavailability of once-daily regimens may limit adherence in children [94,95].

Adherence in adolescents as in adults is influenced by common factors as forgetting doses, complex polymedications, distance to health services, long waiting times for prescription refills, and costs of care [96,97]. However, other challenges including need to be conform, assuming increased responsibility for their own care, difficulties in navigating the health-care system, and lack of links between adult and pediatric services are more specific to adolescent [98,99]. It is estimated that over one-third (38%) of adolescents globally are suboptimally adherent to ART, with substantial regional variation [100].

Many interventions are developed to increase adherence. Peer counselors, mobile phone text messages, reminder devices (alarms, calendar, etc.), cognitive behavioral therapy, and behavioral skill training showed high efficiency to increase adherence [7,101].

4.2. Resistance

There are three main factors associated with the emergence of HIV drug resistances: viral factors (HIV subtype, replication capacity, and preexisting polymorphisms), drug-related factors (drug potency, pharmacokinetics, interactions, tolerance, and genetic barrier to resistance), and program factors (adherence to prescribed ART, drug supply continuity, and retention of patients on treatment) [7]. At least 70% of patients with virological failure have HIV drug resistances, with some studies documenting no resistance at ART initiation [102,103].

4.3. Second-line therapies

Where resistance tests are available, the clinician should identify at least two (preferably three) fully active ARV agents from at least two different classes and assess likelihood of adherence before changing therapy. Substitution or addition of a single drug to a failing regimen is not recommended due to the risk of additional drug resistance and because it is unlikely to lead to durable virologic suppression [14].

Despite some initiatives to get cheaper HIV treatments [104], second-line therapies (Tables 4 and 5) may be unavailable in RLS partly due to the cost, which is three times superior to first-line therapies [105]. If available, the WHO recommends second lines adapted to the first-line therapy (Table 4).

In RLS where resistance tests are not routinely performed, NNRTIs are recommended for infants and young children who experienced virological failure with a PI-based regimen, if it is the only second-line drug available. However, potential re-emergence of archived resistance due to NNRTI exposure during breastfeeding and postnatal prophylaxis may decrease the effectiveness of this regimen [7]. RAL is now an interesting alternative for children older than 4 weeks [82]. If a thymidine analog NRTI drug (AZT or d4T) was used in the failing first-line regimen, a non-thymidine analog NRTI drug (ABC or TDF) should be used in the second-line regimen and conversely. According to some experts, future first-line regimens will all

include DTG but for the time being the experience, and data are still limited in pediatrics [106].

5. Conclusion

Treatment of HIV-infected children evolved extremely rapidly during the last decade. There is an international agreement to start cART in all children infected with HIV independently of clinical signs or CD4 cell count or percentage. New ARV drugs are now available for infants, children, and adolescents that increase the possibilities of different regimens in case of treatment failure or side effects. However, adapted formulations for children and palatability remain limiting factors to improve success of treatment in children. Limited access to new drugs in RLS, where most of the patients are living, will continue to be a great challenge for the next years.

6. Expert opinion

Improving care of children living with HIV modified dramatically their prognosis and quality of life. Children have to live with the different medical and psychosocial constraints of the illness during childhood, adolescence, adulthood, and most probably old age.

The medical staff involved in the management of the disease must have knowledge of the particularities of their patient groups. Some constraints like the number of pills, side effects, or costs of care are shared between all age groups. However, drugs formulation, palatability, or dependence to a caregiver are specific to children. Moreover, the members of the medical staff implicated in these different age groups will have to improve their interaction in order to ensure a successful transition in their different services.

Administration of the most effective, safe, and least toxic cART in the context of lifelong cART is crucial. Moreover, the importance of optimal dosing regimens to limit HIV drug resistance cannot be overemphasized. Many ARVs gained approval based on non-randomized trials investigating safety and pharmacokinetics with extrapolation of efficacy from studies in adults. Implementation of randomized controlled trials to better understand responses to ART throughout the different stages of development are needed to achieve the maximal benefit of managing young patients with HIV infection [107].

Current recommendations are made with the objective to end the AIDS epidemic by 2030. Three targets referred to 90-90-90 are at the heart of the plan. First, 90% of the people living with HIV should know their HIV status. Second, 90% of the people who know their HIV status should receive ART. Third, 90% of the people receiving ART should have suppressed viral loads. In addition to these three targets, elimination of mother-to-child transmission around the world, condom programming, pre-exposure prophylaxis, male circumcision, and adapted services for people who inject drugs are essential. Great efforts are made to reach these objectives in all HIV-infected people, which could stop the propagation of the virus (from the mothers to their child or between partners).

If despite all prevention efforts the child is infected, the goal will be to start a very early treatment to limit the

Table 4. Summary of the WHO-recommended first- and second-line antiretroviral therapy (ART) regimens for children [6].

Children/adolescents	First-line ART regimen	Second-line ART regimen
<3 years	ABC + 3TC + LPV/r AZT + 3TC + LPV/r	AZT or ABC + 3TC + RAL
≥3 years	ABC + 3TC + LPV/r AZT + 3TC + LPV/r	AZT + 3TC + EFV or RAL ABC or TDF + 3TC + EFV or RAL
All ages	ABC + 3TC + EFV/NVP TDF + 3TC/FTC + EFV/NVP AZT + 3TC + EFV/NVP	AZT + 3TC + ATV/r or LPV/r ABC or TDF + 3TC/FTC + ATV/r or LPV/r

Table 5. Summary of the US NIH- and PENTA-recommended second-line antiretroviral therapy (ART) regimens for children [7,12].

First-line ART regimen	Second-line ART regimen US NIH	Second-line ART regimen PENTA
2 NRTIs + NNRTI	• 2 NRTIs + PI or INI	• 2 NRTIs + LPV/r ^a or ATV/R ^b or DRV/r ^c
2 NRTIs + PI	• 2 NRTIs + INI or another PI • INI + PI ± NNRTI ± NRTI	• 2 NRTIs + DRV/r ^c • NRTI + DRV/r ^c + INI • At least three new active drugs
2 NRTIs + INI	• 2 NRTIs + PI • DTG + PI ± 1 or 2 NRTIs	

^a<6 years of age.

^b>6 years of age.

^c≥3 years + ≥10 kg

reservoir. Cure is unfortunately not yet achievable in the absence of extreme therapy such as bone marrow transplantation with HIV-resistant cells (homozygous CCR5 delta 32 allele) used in the 'Berlin patient' [108]. However, there was a hope for cure after the 'Mississippi Baby' [109] who was the first pediatric patient to show long-term viral suppression after being treated between 30 h until 18 months of life with no viral detection and absence of positive antibody test through 30 months of age. But when the child was 46.4 months of age, her viral load rebounded, providing data on how very early treatment may reduce but not eradicate HIV-1 reservoirs [110]. So currently the term 'remission' is preferred to 'cure.'

However, it is now well established that very early treatment lead to shorter time to reach low diversity reservoir size. Early cART in combination with immune-based interventions provide hope in reaching prolonged remission. Current agenda for cure include ongoing studies (Very Early Intensive Treatment of HIV-Infected Infants to Achieve HIV Remission: A Phase I/II Proof of Concept Study (P1115 Plus), Phase I/II Multisite, Randomized, Controlled Study of Monoclonal Antibody VRC01 with Combination Antiretroviral Therapy to Promote Clearance of HIV-1-Infected Cells in Infants (IMPAACT 2008 VRC01), Open-Label, Dose-Escalating, Phase I Study to Determine Safety and Pharmacokinetic Parameters of Subcutaneous (SC) VRC01 and VRC01LS, Potent Anti-HIV Neutralizing Monoclonal Antibodies, in HIV-1-Exposed Infants (P1112 VRC01)) [111] in several age groups (very early ART <48 h of life, early ART <12 weeks of life, late treatment >12 weeks of life and adolescents). For instance, IMPAACT 2008 is a randomized controlled study of monoclonal antibody VCR01 combined with ART to promote clearance of HIV-1-infected cells in infant. Another promising way is the use of broadly neutralizing antibodies (bNAbs) in conjunction of early ART to reach remission. bNAbs to HIV-1 can block acquisition and reach undetectable viral load in chronically infected humanized animal models. In humans, a single infusion of a bNAb (3BNC117) that targets the CD4-binding site on the HIV-1 envelope glycoprotein gp160 led to a quick but temporary reduction in viral loads [112–114].

A parallel intervention that is expected to be successful, especially in the context of early ART and immunotherapy, will be the possibility of short-cycle strategies that could improve adherence particularly in adolescents. The Short-Cycle Therapy in Young People With Chronic HIV-infection (BREATHER) trial compares short cycle therapy (5 days on, 2 days off ART) versus continuous therapy (continuous ART) in patients aged 8–24 years old. This trial shows non-inferiority of keeping virological suppression in children, adolescents, and young adults at 48 weeks, with similar resistance and a better safety profile [115]. Further studies will have to determine if treatment sequences could be administered even with longer intervals in case of HIV remission. Another way of tackling adherence in adolescents is the use of long-acting ARVs such as cabotegravir + rilpivirine for induction and then as maintenance therapy (every 4 weeks or 8 weeks) with an ongoing International Maternal Pediatric Adolescent AIDS Clinical Trials Group (IMPAACT) trial in virologically suppressed, HIV-1-infected children and adolescents, aged 12 to <18 years [116].

For the time being and awaiting for long-term remission or cure for all children living with HIV, we still need to encourage an optimal follow-up in order to hold their quality of life so good as possible. This will only be possible if adapted medical, psychosocial, and pharmaceutical tools continue to evolve and stay available in the different parts of the world.

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