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HAART in HIV-1-infected children: 10 years of clinical experience

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Voor Karel
Voor Anna en Charlotte

Contents

1 General introduction

In the early 1980´s nobody could foresee the tremendous impact of a new clinical entity, now known as `acquired immunodeficiency syndrome´ (AIDS). A large spectrum of clinical manifestations, previously rarely observed, was seen: opportunistic infections, malignancies (Kaposi sarcoma, malignant lymphomas) and neurological disorders (dementia, encephalopathy) (1). In the early days AIDS was predominantly restricted to homosexuals, but subsequently also in hemophiliacs, recipients of other blood products and intravenous drug users and their sex partners. Later on also children, born to mothers with or at risk of the syndrome, were described (2-4). The first patients were seen in the United States of America, but subsequently patients were identified in sub-Saharan Africa. The major symptoms were weight loss and diarrhea and people in rural Uganda called it ' 'slim disease'.

In 1983 the causative agent was identified as a virus belonging to the genus Lentivirus of the Retroviridae (5,6). This virus is now called HIV-1 (Human Immunodeficiency Virus type 1). In 1986 another human homologous virus was identified, nowadays called HIV-2 (7). We will use the word HIV for HIV-1 unless stated otherwise.

Epidemiology

Since the Eighties, a pandemic has emerged all over the world, especially in the developing world, where poverty, poor health care systems and limited resources for prevention and care fuel the spread of the virus. A disproportional burden has been placed on women and children, who in many settings continue to experience high rates of new HIV infections and of HIV-related illness and death (8).

At the end of 2005 the Joint United Nations Program on HIV/AIDS (UNAIDS) / World Health Organization (WHO) Epidemic Update reported that an estimated 38.6 million adults (17.3 million women) and 2.3 million children (< 15 years of age) are now living with HIV, about 4.1 million became newly infected, and an estimated 2.8 million people died of AIDS (8,9) [Table 1]. This is more than 50% higher than the figures projected by the WHO in 1991.

In 2005 globally more than 540,000 children younger than 15 years became infected, about 90% of these infections occurring in sub-Saharan countries being babies born to HIV-positive mothers. The epidemic has left behind 15 million orphans, vulnerable to poverty, exploitation and themselves becoming infected with HIV. In Sub-Saharan Africa, the region with the largest AIDS burden, 2.0 million adults and 330.000 children died of AIDS in 2005.

The HIV incidence rate (annual number of new infections as a proportion of previously uninfected persons) has peaked in some countries (Kenya, Tanzania, Zimbabwe), but in southern Africa the epidemic is still expanding (Botswana, Namibia, Swaziland, South Africa). Women and children are the most vulnerable with a female-male ratio of about 3:1.

TABLE 1 Regional HIV and AIDS statistics and features, 2005 by UNAIDS (8)

Region	Adults (15+) & children living with HIV*	Adults (15+) & children Newly infected with HIV	Adults (15-49) Prevalence (%)	Adults (15+) & child death due to AIDS
Sub-Saharan Africa	24.5 million/2.0 million*	2.7 million	6.1	2.0 million
North Africa and Middle East	440000 / 31000	64000	0.2	37000
Asia	8.3 million / 176000	930000	0.4	600000
Oceania	78000 / 3000	7200	0.3	3400
Latin America	1.6 million / 32000	140000	0.5	59000
Caribbean	330000 / 22000	37000	1.6	27000
Eastern Europe and Central Asia	1.5 million / 6900	220000	0.8	53000
North America, Western Europe and Central Europe	2.0 million / 15000	65000	0.5	30000
TOTAL	38.6 million / 2.3 million	4.1 million	1.0	2.8 million

In Northern America, Western and Central Europe 2.0 million people are living with HIV in 2005, among them 15.000 children younger than 15 years of age. However, in Eastern Europe an estimated 220,000 people were infected with HIV in 2005. Especially in the Ukraine and the Russian Federation epidemics are expanding, forming the biggest AIDS epidemic of Europe. Unsafe intravenous drug practice is the major risk factor in these countries.

In Asia around 8.3 million people are living with HIV − more than two-thirds of them living in India. In China, Indonesia, Vietnam, Bangladesh and Pakistan the HIV prevalence is rising.

In Latin America an estimated 1.6 million people are now living with HIV, among them 32,000 are children younger than 15 years of age.

Mother-To-Child-Transmission

Most infected children acquired their infection from mother-to-child-transmission (MTCT), which can occur during pregnancy, and more often during labor and delivery or during breastfeeding (10-12). In the absence of any intervention the risk of MTCT is 15-30% in non-breastfeeding populations; breastfeeding by an infected mother increases the risk with 15-20% to a total of 30-45% (12).

In 1994 a breakthrough in prevention strategies came by the ACTG 076 study, a placebo-azydothymidine (AZT, zidovudine) controlled study in pregnant HIV-positive women and 6 weeks AZT in their non-breastfed off-spring. AZT reduced the transmission rate by 67%: i.e. transmission rates were observed of 22.6 % in the placebo group and 7.6% in the AZT-treated group (13). The ACTG 076 regimen was rapidly introduced in the Western world, but was too expensive for low-and middle-income countries. Shorter and simpler antiretroviral regimens have subsequently been evaluated in trials in these countries (14-24).

Antiretroviral therapy and also HAART is now given to HIV-infected pregnant mothers in high-income countries, where the MTCT rate has declined to about 1% (25). In the Women and Infants Transmission Study Group (WITS) levels of HIV RNA at delivery and prenatal antiretroviral therapy were independently associated with transmission (25). Recently, concerns have been raised about potential teratogenic effects. The National Study of HIV in Pregnancy in United Kingdom and Ireland showed between 1990 and 2003 no statistically significant association between the prevalence of congenital abnormalities and exposure to ART overall: 3.4% (90 of 2657 pregnancies) in exposed pregnancies and 2.2% (10 of 463 pregnancies) in non-exposed pregnancies (p=0.17); prevalence was similar whether or not exposure to whatever type of ART had occurred in the first trimester (p=0.48) (26).

HAART before and during pregnancy has been associated with prematurity, pre-eclampsia and gestational diabetes (27-32). Women may already be at increased risk of nevirapine-associated hepatotoxicity, especially those with CD4+ T cells > 250 cells/mm^3 (31).

Elective cesarean section (ECS) is an efficacious intervention for the prevention of MTCT among HIV-1-infected women not taking antiretrovirals or taking only zidovudine, but the risk of postpartum mortality with ECS is higher than that associated with vaginal delivery, yet lower than with non-ECS (33-35).

Long-term effects of maternal HAART in non-infected HIV-exposed children have been observed as well. Bunders et al found alterations in hematological parameters, which may persist for a long period (36). To date, the clinical implications remain uncertain.

A French group described neurological involvement in HIV-and ART-exposed infants, possibly associated with mitochondrial disease (37). This association has not been confirmed in other large cohorts in the US or by the European Collaborative Study on MTCT.

In resource-constrained settings much effort focuses on the implementation of HIV-testing and counseling during pregnancy and introduction of more effective antiretroviral regimens, starting during the third trimester in HIV-infected pregnant women (38,39). However, in 2005 only 9% of the pregnant women received ART (39). Although reducing MTCT assessed at 4-6 weeks post-partum to 2-4%, infants remain at risk when the mothers continue breastfeeding (39). Research is ongoing to evaluate several new approaches to prevent HIV transmission during breastfeeding (39,40).

The reverse side of this is an unjustified or half-hearted use of ART. As a consequence, viral resistance may emerge on large scale and limit future treatment options for both mothers and children (41,42).

Diagnostic tests in pediatric HIV and immunophenotyping

Early diagnosis of HIV-infection in vertically HIV-exposed children is hampered by trans-placental maternal HIV antibodies. Virological assays, including PCR tests to detect HIV RNA (or DNA) or to quantify the viral load, can be used to determine the presence of HIV or rule out infection in infants less than 18 months of age. Serologic diagnostic methods, including HIV-specific ELISA, immunofluorescence, and western blot assays, can be

used to diagnose HIV in infants over 18 months of age, when maternal antibodies have disappeared completely from HIV-exposed infants (43).

The challenge of the early and accurate diagnosis of perinatally HIV-exposed infants is the use of new assays to detect different HIV subtype infections that are prevalent in developing countries. Rapid, simple, and inexpensive serologic and virologic assays are being developed for worldwide use (43).

Dried paper blood spots have already been used with PCR tests for HIV RNA and have been shown to be very reliable. Recently, a quantitative p24 antigen test has been developed using dried paper spots of blood drops, which showed similar sensitivity and specificity to tests using blood plasma, has the potential to further simplify testing and improve health care delivery to HIV-affected individuals in resource-constrained countries (44).

Immunophenotyping is performed by flowcytometry and routinely used to count the T cells (subdivided into the major subsets of CD4+ T helper cells and cytotoxic CD8+ T cells), CD19+(CD20+) B cells and NK cells. We believe that it is better to use absolute CD4+ T cell counts in pediatric studies on T cell repopulation during HAART, since CD4+ T cells as percentages of total T-cell counts are influenced by the major changes in the number of CD8+ T cells, a condition often encountered in HIV-infected patients.

In the pediatric population we meet the problem that CD4+ T cell counts change with age. Reference values are much higher in infants and young children than in older ones. Therefore, the absolute CD4+ T-cell counts were calculated as percentage of normal absolute values resulting in an independent age-adjusted parameter for the degree of T cell restoration (45,46).

Natural history and classification of disease

Before HAART the natural history of HIV/AIDS in children showed a much more rapid progression with a high viral load, a more profound immune deficiency (depletion of CD4+ T cells) and impaired growth characteristics. Around 23% of HIV-infected infants developed AIDS before the age of 1 year, and nearly 40% by 4 years of age. Ten percent died in their first year of life and almost 30% before reaching the age of 5 years (47).

Barnhart described the natural history of pediatric HIV infection, using five progressive stages using the clinical categories in the CDC 1994 pediatric HIV classification system (48): stage N, no signs or symptoms; stage A, mild signs or symptoms; stage B, moderate signs or symptoms; stage C, severe signs or symptoms; and stage D, death.

A total of 2,148 perinatally HIV-infected children, born between 1988 and 1993, were included in the analysis. The estimated mean times spent in each stage were: N, 10 months; A, 4 months; B, 65 months; and C, 34 months. The authors estimated that a child born with HIV infection has a 50% chance of severe signs or symptoms developing by 5 years of age and a 75% chance of surviving to 5 years of age. For a child in stage B, there is a 60% chance of severe signs or symptoms developing within the next 5 years and a 65% chance of surviving 5 more years. The estimated mean time from birth to stage C was 6.6 years (95% CI, 5.7-7.5 years), and the estimated mean survival time was 9.4 years (95% CI, 8.1-10.7 years) (49).

To date, in children 3 groups of children could be distinguished: 'rapid progressors', 'intermediate progressors' and 'slow progressors' (about 20%, 60% and 20% of infected children, respectively) (50-53). Due to due to serious impaired immunity the 'rapid progressors' often present with opportunistic infections, such as Pneumocystis jiroveci (previously called Pneumocystis carinii) pneumonia (PCP) (54), extensive cytomegalovirus infection, and recurrent oral and esophageal Candida spp infection. These children may also present with psychomotor developmental delay or arrest and serious neurological signs such as spastic tetra paresis due to progressive or static HIV-encephalopathy (55-57). Growth retardation and failure-to-thrive are very striking features in this group of children and growth seemed to be one of the most sensitive indicators of disease progression in children with AIDS and the absence of growth indicated a poor prognosis, even in children treated with antiretroviral therapy (58,59). Other indicators consist of a high or progressively increasing HIV load and CD4+ T cell depletion (60,61). The "intermediate progressors" may present with milder symptoms, like recurrent upper- and lower respiratory infections, lymphadenopathy, hepato(spleno)megaly and milder growth retardation.. The group of "slow progressors" consists of children with very mild symptoms and therefore sometimes delayed diagnosed, when they have already passed their first decade. The Centers for Disease Control and Prevention (CDC) developed a Classification system, based on the severity of clinical symptoms (N, A, B, C) and immune deficiency (I, II, III). This Classification was revised in 1994, when lymphoid interstitial pneumonia (LIP) changed into a B instead of a C classification item (48).

Treatment and outcome measures

In 1987 AZT became available. Also children were treated and the effects on neurological manifestations like HIV-encephalopathy and on LIP were remarkable (55,56). In the early 1990´s more nucleoside reverse transcriptase inhibitors (NRTIs) became available. In 1993 it became obvious that mono therapy was inferior to combination therapy (62). In 1995 it became possible to quantify the HIV RNA load by amplification methods, the so called HIV Polymerase Chain Reaction (HIV-PCR) (61). From that time onward, the effectiveness of antiretroviral therapy (ART) could be monitored virologically It became also clear that the "virologic set point" in children was much higher than in adults (64). In 1996 an important new class of drugs was introduced namely the protease inhibitors (PIs) (65). The heydays of HAART started. In 1997 the first PIs in children were registered and less mortality, less morbidity and less hospitalization were observed in infants and children treated with these combination therapies (66-70). In 1997 the first infants and children of our Pediatric Amsterdam Cohort on HIV (PEACH) started HAART. Long-term experience with combination antiretroviral therapy that contains nelfinavir for up to 7 years in this cohort is described in Chapter 2.

At that time the idea was "hit hard, hit early". There were no worries about toxicity and long-term effects and scientists believed that with HAART one could eradicate HIV in 3 years. In 1998 sobering started and people realized that this was impossible and that HIV was harbored at sanctuary sites in the body (71) and the first side effects were noticed

like lipodystrophy, metabolic abnormalities and mitochondrial toxicity (72-74). This was ascribed not only to the PIs, but also to NRTIs. Guidelines in adults changed and a more conservative approach as to whether and when to start HAART was initiated. Adherence appeared to be the Achilles heel of successful suppression of HIV and one of the most important factors in virologic failure in adults as well as in children (75). Simplification of regimens may facilitate a better adherence (76,77). In 2002 we commenced a once-daily HAART regimen containing efavirenz and 3 NRTIs (abacavir, ddI, and 3TC) to increase compliance and virologic success rates in HIV-infected children. The safety, tolerability and effectiveness of this once-daily regimen for up to 2 years are described in Chapter 3.
Infants and children have a developing immune system in which the thymus has an active participation. Immediately after birth, neonates have high numbers of CD4+ and CD8+ T cells, all of which are naïve. Children have to face many infections in the first years of their life; numbers of activated and memory CD4+ and CD8+ T cells increase progressively toward adult values (78). IgA (and to a lesser extent IgG) is transferred through mother milk to the neonate. Only maternal IgG is actively transported over the placenta to the fetus during the second and third trimesters of pregnancy (78). These antibodies partly protect the neonate to infections. However, the HIV-specific antibodies have not been proven to protect neonates from perinatal infection upon HIV exposure. After the first one to two years of life, children have generally developed their own humoral adaptive immune system against most exogenous antigens. Cellular immunity already matures slightly earlier. Most apparently, HIV infection impeaches on the natural maturation of the adaptive immune system enormously. Children with profound immune suppression develop AIDS-related illness during the first months of life. Without treatment few of these children will survive more than 2 years.
One of the goals of HAART is to reconstitute the HIV-induced immunodeficiency. The so-called immune reconstitution in HIV-infected children upon start of HAART is described in Chapter 4. On the other hand, persistent humoral immune defect in HAART-treated children were found toward vaccination, both against primary as well as booster immunizations, as is described in Chapter 5.
Interaction of antiretroviral drugs and co-medication may occur for several reasons, sometimes because of their clearance by the same metabolic pathways, redistribution, induction of enzyme systems, etc. These interactions can be relevant for ART drug levels as well as medication-related toxicity. Measuring the plasma levels of certain medication may be indicated. Infants and children should be monitored by measuring antiretroviral drug levels because they are growing and developing individuals with considerable intra-patient and inter-patient variability (79). Recently Menson et al reported a prominent underdosing of antiretroviral drugs in HIV-infected children in the UK and Ireland (80). Pharmacokinetics of nelfinavir and its active metabolite M8 in HIV-infected children is described in Chapter 7 and 8. As an example of an unforeseen interaction of medication and toxicity, we have described a case of liver failure in a child receiving HAART and voriconazole in Chapter 9.

TABLE 2 Metabolic complications of ART in children (80)

Metabolic complications	Mitochondrial toxicity [#]& lactic acidemia	Dyslipidemia[•]	Lipodystrophy syndrome [*]& cardiovascular disease	Renal toxicity [a]	Liver toxicity [b]	Bone disease [§]	Insulin resistance[°]
Class							
NRTIs							
abacavir	HSS	?	?	–	+	?	?
AZT	+	?	?	–	+	?	?
D4T	+	+	++	–	+	±	?
ddI	+	?	+	–	+	?	?
3TC	+	±	±	–	+	?	?
tenofovir	±	±	?	+	?	+	?
NNRTIs							
nevirapine	–	–	–	–	+	–	–
efavirenz	–	±	–	±	+	–	–
PIs							
nelfinavir	–	+	+	–	+	+	±
indinavir	–	+	+	+	+	+	±
ritonavir	–	+	+	–	+	+	±
atazanavir	–	+	+	–	+	?	?
saquinavir	–	+	+	–	+	+	±
lopinavir/ ritonavir	–	+	+	–	+	+	±

[#] described in HIV-infected and HIV-exposed children
[•] total cholesterol ↑, low density lipoprotein cholesterol (LDL) ↑, triglycerides (TG) ↑, high density lipoprotein cholesterol (HDL) ↓
[*] lipodystrophy syndrome encompasses changes in fat distribution typically manifesting as lipoatrophy, with or without central adiposity, frequently associated with alterations in lipid regulation and glucose homeostasis
[a] renal tubular dysfunction in adults described (tenofovir), crystalluria (indinavir)
[b] liver transaminases ↑
[§] osteopenia / osteoporosis (phosphate ↓), or osteonecrosis (possibly related to other factors)
[°] reduced insulin sensitivity occurs naturally in puberty
\+ possible contribution or in combination with other factors like HIV itself, certain hormones, immune reconstitution
± less likely to occur
– unrelated or as yet not suggested
? unknown
HSS hypersensitivity syndrome (i.e. rash, nausea, vomiting, diarrhea, coughing, lactic acidemia)

Future treatment perspectives

Simplification of HAART and fixed-dose combinations is needed in the developed countries as well as in the developing world. The initiatives to produce cheaper generic antiretroviral drugs, by preference in compound tablets or suspensions, should be encouraged.

After the introduction of ART the natural history of HIV-infected children has changed with a dramatic decrease in morbidity and mortality. These children now become adolescents and young adults who have to cope with problems of adherence and long-term side effects of persisting life-long infection and the use of antiretroviral drugs (81).

The Pediatric Amsterdam Cohort on HIV (PEACH) was established to optimize ART in HIV-infected children and to create the possibility to study different aspects of HIV/AIDS and treatment. The implications of viral co-infections before and during ART and the long-term follow-up data of this cohort made it possible to obtain insight into clinical, virologic and immunologic aspects of HAART in children of different ages and background in daily clinical practice. In this cohort we were also able to perform pharmacokinetic studies of different antiretroviral compounds and to develop a protocol for once-daily therapy that has been further improved by implementation of a form of directly observed therapy (DOT).

Only by our efforts to guarantee the highest adherence possible we may offer the best chances of long-lasting success in HIV-infected children, while reducing the foreseeable long-term side effects to the minimum [Table 2] (82). After all, an HIV-treating pediatrician remains a family doctor, even though specialized to a high degree on the treatment of −what is generally appreciated as− one infectious disease.

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2 Long-term experience with combination antiretroviral therapy that contains nelfinavir for up to 7 years in a pediatric cohort

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Abstract

Objective We sought to provide long-term data on the clinical, immunologic and virologic response to highly active antireteroviral therapy (HAART) in infants and children who are naive to protease inhibitor (PI).

Methods HIV-1-infected children, naive to PIs, were treated with a combination of nelfinavir and 2 nucleoside reverse transcriptase inhibitors (NRTIs; stavudine and lamivudine) in an observational, prospective, single-center study. Virologic failure-free survival was assessed by Kaplan-Meier analyses. The increase in $CD4^+$ T cells during follow-up was estimated with a generalized linear model incorporating repeated measurements.

Results Thirty-nine HIV-1-infected children were included and followed for a median period of 227 weeks (IQR 108 - 275). The virologic failure-free survival rate was 74%, 66%, 58% and 54%, after 48, 96, 144, and 240 weeks, respectively. Children who experienced virologic failure in 48 weeks (or 96 weeks) were younger at baseline compared with the responders (0.8 versus 5.3 years; $p<0.003$). Eighteen children remained on the regimen for > 5 years. All children, including the non-responders, showed a sustained immunologic response. Grade 3 to 4 toxicity was observed in 2 patients only. Eleven developed clinically evident lipodystrophy.

Conclusion Combination therapy can be used safely in infants and children over a long period. Young age is strongly associated with virologic failure. Although the virologic response declined, immunologic parameters and clinical improvement were sustained up to 7 years, at the expense of lipodystrophy.

Introduction

Since the Food and Drug Administration approval of nelfinavir, indinavir and ritonavir for children in 1997, the first trials in a limited number of children showed virologic and immunologic improvement.[1-3.] Mortality, disease progression and hospital admissions in HIV-infected children have declined substantially since the introduction of highly active antiretroviral therapy (HAART), just as has been seen in adults.[4-6] In adults, it was shown that most patients had changed their first regimen after 4 years of HAART because of virologic failure and the availability of alternative drug regimens. In adults a continued increase in $CD4^+$ T cell count was seen in patients who experienced sustained virologic suppression.[7,8] However, one can not extrapolate results in adults to children because of differences in immunity (*e.g.*, the immaturity of the immune system and larger thymic output); in pharmacokinetics and pharmacodynamics of antiretroviral drugs in infants and children; and, most important, in formulation, availability of drugs and strict adherence to therapy. Studies have shown that the age-adjusted $CD4^+$ T-cell numbers increase in infants and children, especially in the more immunocompromised ones, even when failing in viral suppression.[9] Despite reasonably good virologic response rates at 48 and 96 weeks of HAART, data from several pediatric studies have shown that the virologic response in children is less prominent compared to adults.[1,3,9-14]

Infants and children often start antiretroviral therapy at very young ages and have to use their medication lifelong. Hence, there is an urgent need for more long-term data on virologic; immunologic; and clinical response to HAART in children. The rationale for this study was to evaluate the long-term virologic, immunologic and clinical, especially growth, effectiveness and safety of a combination antiretroviral therapy that contains nelfinavir, lamivudine and stavudine in children who were included in the Pediatric Amsterdam Cohort on HIV (PEACH).

Methods

Patients

The Pediatric Amsterdam Cohort on HIV-1 (PEACH) consists of children and young adolescents who are younger than 18 years. Since 1997, patients have received highly active antiretroviral therapy. Current American and European treatment guidelines for HIV-1 infection in children recommend the use of 2 nucleoside reverse transcriptase inhibitors (NRTIs) in combination with either a protease inhibitor (PI) or a nonnucleoside reverse transcriptase inhibitor (NNRTI).[15,16] According to the history of antiretroviral therapy, some children in PEACH had initially been treated with azidothymidine (AZT), followed by AZT combined with dideoxyinosine (ddI) or dideoxycytidine (ddC), until the introduction of nelfinavir (NFV) as the first PI available for children. Because of the previous use of certain NRTIs, NFV was combined with stavudine (d4T) plus lamivudine (3TC).

Between September 1997 and January 2005, a prospective, observational study was performed. Inclusion took place until January 2002. Untill then, of the 48 children in follow-up, 39 children were included in the study using the NFV-containing treatment regimen. HIV-1-infected children were eligible, when they were aged 3 months to 18 years, and had a plasma viral load (pVL) of > 5000 copies/mL (mean of 2 measurements in < 4 weeks) and/or $CD4^+$ T cell counts < 1750/μL for those who were younger than 1 year, < 1000/μL for those who were 1 and 2 years, < 750/μL for those who were 3 and 6 years, and < 500/μL for those who were older than 6 years. Previous exposure to AZT, ddC or ddI was allowed. There were no restrictions with regard to ethnicity, gender, route of HIV acquisition, or disease stage. Nine children were excluded, because they did not meet the inclusion criteria. Five were immunologically stable and did not start any antiretroviral therapy. Four children in the cohort started another regimen during the inclusion period. The Medical Ethical Committee of our institute approved the protocol. Parents or caregivers gave written informed consent.

Medication

Patients received d4T (1 mg/kg twice daily as oral solution or capsules) plus 3TC (4 mg/kg twice daily as liquid formulation or tablets) plus NFV (30 mg/kg 3 times daily or 45 mg/kg twice daily as pediatric formulation (50 mg NFV per gram of powder or as tablets) .[17] Children who were able to swallow capsules received the NFV tablets and smaller children were using the NFV powder dissolved in water or milk or crunched tablets in some custard. Dosage adjustments were performed according to the weight of the children and, in case of NFV, consecutive plasma levels. It was recommended that the children take their regimen with food.

Protocol

At each visit physical examination was performed, including weight, length and head circumference measurements. The same 2 physicians clinically diagnosed lipodystrophy during the study. Independent scorings were made and were considered clinically evident when both agreed. Blood was drawn before; at 1 and 2 weeks; and 1, 2 and 3 months after initiation of HAART and every 3 to 4 months thereafter. At each visit NFV levels were analyzed to adjust dosing when necessary.

Lymphocyte subsets were analyzed with the FACScan (Becton Dickenson Immunocytometry Systems, San Jose, CA). Age correction for $CD4^+$ and $CD8^+$ T cells was done by dividing the counts by the mean of an age matched healthy control group.[18]

From 1997 to 2000 pVL was routinely measured using NucliSens HIV-1 QT (bioMérieux, Boxtel, the Netherlands) with a lower limit of quantification (LLQ) of 400 copies/mL. From 2001-2005 pVL was measured using Versant HIV-1 bDNA 3.0 (Bayer, Mijdrecht, Netherlands) with a LLQ of 50 copies/mL (input 1 mL of plasma).

Virologic failure was defined as 2 consecutive pVL > 1000 copies/mL after a pVL < 400 copies/mL. Patients who never reached a pVL < 400 copies/mL, were defined as failing at the first measurement that was higher than the previous one after an initial decline in pVL (pVL nadir).

Adverse events were recorded during the study period and defined as any clinical sign or symptom or meaningful laboratory test abnormality that was possibly or probably related to the study medication, excluding HIV-related disorders. The National Institute of Allergy and Infectious Diseases (Division of AIDS) toxicity table was used for grading severity of pediatric adverse events. Parents were asked for the presence of anamnestic adverse events at every visit.

We analyzed the growth of the children by means of the *z* scores (standard normal deviation) of weight and height. These scores were calculated with the use of the Growth Analyser 2.0 software (Dutch growth foundation, Rotterdam, Netherlands) using Dutch reference values.

Statistics

The primary outcome measure was virologic failure-free survival, which was assessed using Kaplan-Meier analysis. Censoring was applied when the last patient visit or a switch to a simplified regimen occurred before virologic failure. The secondary outcome measures were factors that were associated with virologic failure, changes in $CD4^+$ and $CD8^+$ T cells over time, changes in growth parameters (weight, height) over time and reported adverse events. The mean age-adjusted $CD4^+$, $CD8^+$ T cells (age correction for $CD4^+$ and $CD8^+$ T cells was done by dividing the counts by the mean of an age-matched healthy control group.[18]), and height and weight *z* scores were modeled using a mixed model that incorporated repeated measurements. This model handles missing data adequately by estimating the outcome given a specific covariate structure. The estimates of a specific level of the fixed effects were modeled using the 'first order autoregressive' approach. Differences in these estimates between different levels of the variable were tested for significance using t statistics. Success or failure of treatment after 24 weeks was added to all models as a time-dependent variable. Where subgroups of patients are compared, the differences between groups were evaluated using the Fisher's exact test for categorical data and the Kruskal Wallis test for continuous data. All statistical analyses were performed using SPSS for Windows version 11.5 (SPSS, Chicago, Il). A 2-sided p-value < 0.05 was considered statistically significant.

Results

All 39 HIV-1-infected children who started antiretroviral treatment with d4T, 3TC, and NFV between September 1997 and January 2002 were included in the present analyses. Baseline characteristics are shown in Table 1. Sixteen (41%) children had been pretreated with 1 or 2 NRTIs (AZT, ddI, or ddC) for a median of 179 weeks before enrollment (interquartile range (IQR) 104 – 310 weeks). The median age of the children at baseline was 4.7 years (IQR: 1.1 - 8.8 years). Thirty-four (87 %) children acquired HIV infection perinatally from their HIV-1-infected mother, 16 (41%) children presented with CDC-C classified AIDS defining symptoms. The majority (69%) of the children were black

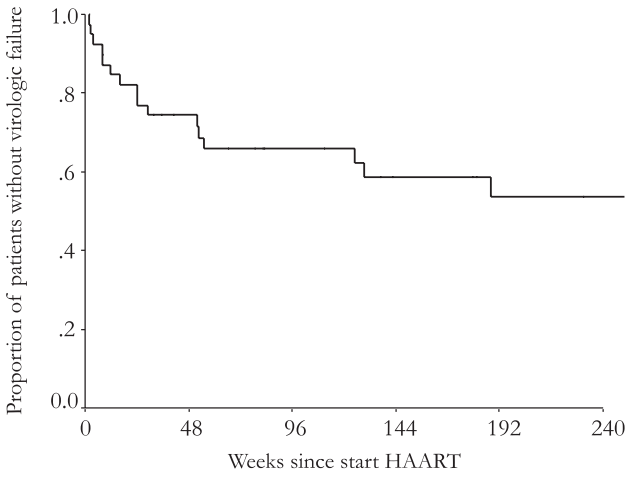
(African/Surinamese), whereas 18% were white 10% were mixed/Caribbean, and 3% were Asian. The children were on study medication for a median duration of 185 weeks (IQR 69.5 - 264.9 weeks).

The study medication had to be discontinued in 26 (69%) children during the follow-up for the following reasons: virologic failure (n=16), major toxicity (n=2; diabetes mellitus and high cholesterol, both with complete recovery), poor palatability and refusal (n=1), and switch because of simplification of therapy (n=4). Although routinely assessed, other grades 3 to 4 toxicity adverse events were not reported. Three were lost to follow-up. One child initially started with the study medication but nevirapine was added to the regimen because of a very high pVL (> $5x10^6$ copies/mL), but once HIV-RNA reached undetectable levels, NFV was stopped after 20 weeks.

Virology

At baseline, the median pVL for the whole group was 4.9 $\log_{10}$ copies/mL (IQR 4.4 - 5.4 $\log_{10}$ copies/mL). There was no significant difference between the naive and pre-treated patient groups. The median time to reach undetectable pVL was 7.6 weeks (IQR: 2.2-12.6). Of the patients for whom therapy failed or study medication was discontinued at any time during the follow-up of 240 weeks (n=29), 7 never had a pVL below the LLQ. These were young (median age 0.7 years (IQR 0.3-1.0)). Of the remaining 32 children (median age 5.3 (IQR 3.0-9.4)) in this observational cohort, 22 showed a rebound of their pVL after having had a period of viral suppression below the LLQ. Eight of 22 patients whose pVL had become undetectable during treatment but were subsequently failing, did so in the first year of therapy (Table 2). Children who experienced virologic failure at 48 and 96 weeks

FIGURE 1 Kaplan-Meier survival analysis of time to virologic failure. Number of patients at risk at start and after 1, 2, 3, 4 and 5 years are indicated. Censoring was applied if the last patient visit or a switch to a simplified regimen occurred before virologic failure.



Weeks since start HAART	0	48	96	144	192	240
# of patients at risk	39	26	19	14	11	10

TABLE 1 Baseline characteristics of children who started with NFV-containing regimen and comparison between pretreated and antiretroviral naive children

	Total	Naive	Pre-treated
Number of patients	39	23	16
Female	21 (54%)	12	9
Age, yrs[1]	4.7 (1.1- 8.8)	4.3 (0.8-7.1)	5.3 (2.7- 8.8)
CDC- C[2]	16 (41%)	11	5
Route of transmission:			
MTCT[3]	34 (87%)	20	14
sexual	5 (13%)	4	1
Race			
black	27 (69%)	17	10
non black	12 (31%)	7	5
Duration pretreatment median, wks (IQR)		0	179 (104-310)
CD4+ T cells, abs per µL[1,4]	470 (140 -850)	550 (180-1010)	440 (50- 700)
CD4+ T cells, %[1]	17 (11- 23)	20 (13-30)	15 (3-19)
CD4+ T cells, age adjusted[1]	0.33 (0.08-0.51)	0.35 (0.17- 0.52)	0.32 (0.04-0.5)
CD8+ T cells, abs per µL[1]	1230 (750-1980)	1270 (800-1970)	1230 (380-2230)
CD8+ T cells, %[1]	50 (32- 61)	50 (33- 63)	49 (29- 60)
CD8+ T cells, age adjusted[1]	1.21 (0.81-1.94)	1.17 (0.83-1.94)	1.38 (0.35- 2.46)
HIV-1-RNA log copies/mL[1]	4.9 (4.4-5.4)	5.0 (4.5-5.8)	4.8 (4.4 - 4.9)
Height–for–age[1]	–1.08 (–2.26 to –0.58)	–0.87 (–2.26 to–0.58)	–1.42 (–2.34 to–0.36)
Weight–for–height[1]	–0.28 (–0.99 to +0.48)	–0.47 (–0.96 to +0.45)	0.19 (–1.39 to+0.77)

[1] median, interquartiles between brackets (IQR), [2] CDC-C: HIV pediatric Classification by the Centers for Disease Control and Prevention. MMWR 1994;43:1-19, [3] MTCT: mother to child transmission, [4] CD4+ T cells, abs per µl: absolute numbers of CD4+T cells per µl

on HAART after an initial period of successful virologic suppression, were younger at the start of HAART compared with those without virologic failure (median 0.8 vs. 5.3 years (p=0.003), at 48 weeks and 1.0 vs. 4.8 years at 96 weeks (p=0.098)).

Sixteen children with virologic failure continued study medication after failure occurred for a median period of 3.3 years (range 0.3 - 6.5 years). Reasons to continue the failing antiretroviral regimen were the presence of stable $CD4^+$ T cell counts and a stable clinical condition without any deterioration. All patients had stopped trimethoprim-sulfamethoxazole prophylaxis. These children had developed antiretroviral drug resistance mutations and alternative drugs were not available at that time. Later, appropriate switches to second-line HAART regimens could be made successfully.

Immunology

At baseline, the median $CD4^+$ T cell count for the total study population was 470/µL (IQR: 140 – 850/µL) and adjusted for age 0.33 (IQR: 0.08 - 0.51). In relative terms to the total number of lymphocytes, the $CD4^+$ T cell percentage was 17% (IQR: 11 - 23). The baseline $CD4^+$ T cell percentage was significantly lower in children who were pretreated

TABLE 2 Number of patients on HAART, virologic response and failure, reasons to stop and lipodystrophy.

Weeks after start	0	24	48	72	96	144	192	240	288	336
Years after start			1		2	3	4	5	6	7
A. Number of children on treatment	39	38	34	32	30	27	22	18	8	5
Nr on HAART with success		32	26	22	19	14	11	10	5	2
Nr on HAART after failure		6	8	10	11	13	11	8	3	3
B. Reason to stop:										
Virologic failure			1		1	1	5	2	5	1
Lost to follow-up					3					
Grade 3 or 4 toxicity			1			1				
Switch therapy[1]			2			1		1		
Intolerance			1							
C. Lipodystrophy			1			2	3	5		

[1] while undetectable, simplification

(15%), compared to children who had not received previous antiretroviral medication (20%). The median $CD8^+$ T cell counts for the total study population was 1230/µL (IQR: 750 – 1980/µL) and adjusted for age 1.2 (IQR 0.8 - 1.9).

The median age-adjusted $CD4^+$ T cell counts demonstrated an increase in the first 48 weeks of treatment (Fig. 2A), which was similar for the children who had a virologic failure and those who had not (p=0.95; Fig 2A, insert). The age-adjusted absolute $CD8^+$ T cell counts and the $CD8^+$ T cell percentage demonstrated a slight but nonsignificant decrease in the total study population as well as in the subgroups based on virologic response (Fig. 2B).

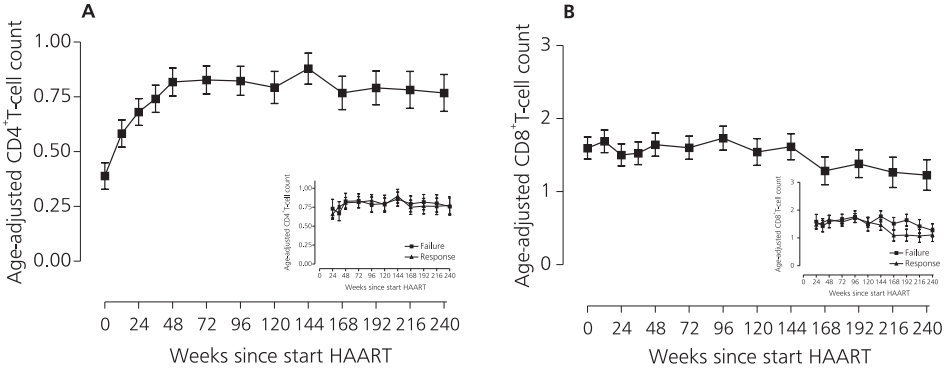


FIGURE 2A. Age-adjusted $CD4^+$ T-cell count during 240 weeks follow-up on HAART. Follow-up of all patients during treatment with NFV-containing regimen. In the insert, a comparison is shown between children with undetectable pVL and children that failed on therapy. No difference over time was found between the groups. Interaction term (time*virologic success), p=0.9. Bars indicate standard errors of the mean.

FIGURE 2B. Age-adjusted $CD8^+$ T-cell count during 240 weeks follow-up on HAART. Follow-up of all patients during treatment with NFV-containing regimen. In the insert, a comparison is shown between children with undetectable pVL and children that failed on therapy. No difference over time was found between the groups. Interaction term (time*virologic success), p=0.9. Bars indicate standard errors of the mean.

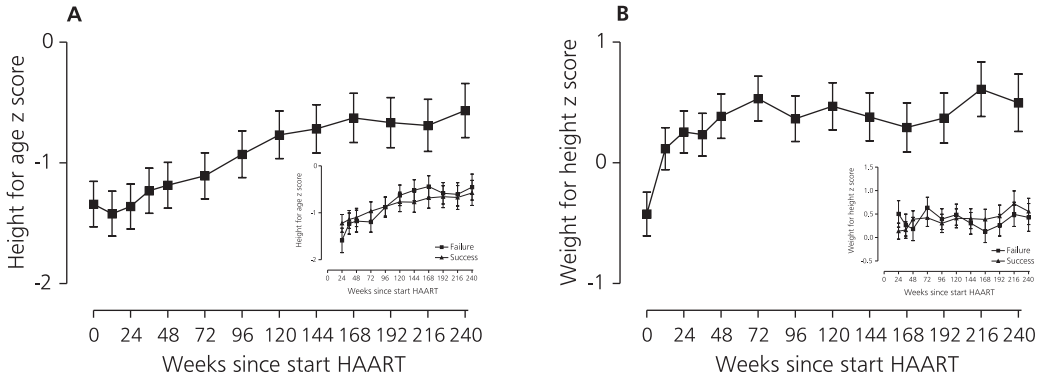


FIGURE 3A Height-for-age z scores during 240 weeks follow-up on HAART. Z scores were calculated for each measurement of height according to age and gender using the 1997 Dutch reference curves. Follow-up of all patients during treatment with NFV-containing regimen. In the insert, a comparison is shown between children with undetectable pVL and children that failed on therapy. No difference over time was found between the groups. Interaction term (time*virologic success), p=0.5. Bars indicate standard errors of the mean.

FIGURE 3B Weight-for-height z scores during 240 weeks follow-up on HAART. Z scores were calculated for each measurement of height according to age and gender using the 1997 Dutch reference curves. Follow-up of all patients during treatment with NFV-containing regimen. In the insert, a comparison is shown between children with undetectable pVL and children that failed on therapy. No difference over time was found between the groups. Interaction term (time*virologic success), p=0.6. Bars indicate standard errors of the mean.

Disease progression and toxicity

None of the children developed an AIDS-defining illness or died while on study medication. Clinically evident lipodystrophy was seen in 11 (28%) children after a median of 49 months (range 10 - 83): 9 with lipoatrophy; of these 9 children, 2 in combination with an adipose trunk (1 of these 2 was pretreated extensively for 305 weeks and developed lipodystrophy within the first year of HAART) and 2 in combination with a buffalo hump; of 2 additional children out of the 11, 1 with a solitary adipose trunk and 1 with a solitary buffalo hump. In 2 of these 11 children pVL stayed undetectable for 7 years; the others failed due to nonadherence.

Growth and development

Growth parameters are shown in Figure 3A and B. The median height-for-age *z* score at baseline for the total study population was –1.08 (IQR −2.26 to −0.58), and the median weight-for-height *z* score was –0.28 (IQR –0.99 to 0.48). There were no statistically significant differences between naive and pre-treated children at baseline.

After the first year of HAART, the height-for-age *z* scores gradually increased to a plateau but never reached the mean of the general mixed Dutch population, which by definition is 0 (Fig. 3A). Height-for-age was significantly higher than baseline from week 96 onward. In the first year of HAART, there was a remarkable increase in weight-for-height *z* scores. The increase was mainly seen in the first 24 weeks after the start of HAART from median −0.3 to 0.5 (Fig. 3B). Comparing virologic responders and nonresponders during follow-up, we did not observe significant differences in height-for-age *z* score and weight-for-height *z* scores with regard to baseline results over time (p=0.50, p=0.57, respectively).

Discussion

We demonstrated in the present analyses that a NFV-containing regimen for up to 7 years is feasible and effective to some extent. Of the 39 included patients, 18 were on the initial regimen after a follow-up of 240 (~ 5 years) and 5 after 336 weeks (~ 7 years). The virologic failure-free survival rate at 5 years of follow-up was 54%. All children showed an adequate increase in $CD4^+$ T cells, regardless of virologic failure. The frequency of reported grade 3 to 4 adverse events was low. After start of HAART, the growth of these children slowly but progressively improved.

The reported virologic response rate did not differ from other studies in children.[9-12,19-22] Studies on NFV in combination with 2NRTIs have shown viral response rates (intention-to-treat) of 69% at < 400 and 44% at < 50 copies/mL, and, when combined with an additional NNRTI, ~ 80% at < 400 and 63% at < 50 copies/mL after 48 weeks, respectively.[10,11,22] Our study population is small (n=39) but the follow-up of this cohort using NFV-containing HAART, is over an extended period of time

Children with virologic failure at 48 and 96 weeks were younger at the start of HAART. The relation between virologic failure and age at start of HAART was reported earlier by Walker et al.[13] One explanation could be that younger children were initially dosed for NFV according to the manufacturer's instructions, which turned out to be too low.[17,23,24] However, drug levels in these young children were not very low to absent and recent data from the 2NN study group (the regimen contains 2 NNRTIs) in adults suggest that drug levels in therapy-adherent patients have a poor sensitivity to predict virologic failure.25 This may hold true for pediatric cohorts as well. A recent analysis indeed demonstrated early viral decay rates in HIV-infected children starting with HAART with a median of 2.1 days (IQR 1.8 - 3.0), similar with adults.[26] Importantly, there was no difference in baseline pVL between the treatment-naive and pretreated children. This makes a biological basis for the relation between age and virologic failure unlikely and makes non-adherence probable as an explanation for virologic failure at very early age.

Immune reconstitution occurred irrespective of virologic response, indicating that HIV-1-infected children have a greater capacity to sustain lymphocyte numbers compared to adults, even in the presence of virologic failure. Studies in adults have demonstrated that restoration of functional immunity correlated with increases in the number of naive T cells, reflecting a critical role of the thymus.[27] Because of an intact thymus, children have a greater capacity to restore immunity as indicated by their rapid $CD4^+$ T cell recovery upon initiation of HAART.[28,29]

At baseline, there was no significant difference in growth-related parameters (height-for-age, weight-for-age, and weight-for-height) between naive and pretreated children. Whereas Chantry et al.[30] demonstrated the short-term beneficial effect of NRTIs on height, weight and head circumference, in our cohort the pretreated children had not profited in this respect from the previous use of antiretroviral therapy. In the first year on HAART, there was a remarkable increase in weight-for-height *z* score.

With respect to toxicity, only 2 patients had to stop the study medication because of adverse events (diabetes and high cholesterol). However, long-term follow-up demonstrated a high prevalence of lipodystrophy, especially in those children with longer use of the study medication, as was recently reported in children by Sanchez Torres et al as well.[31] We already observed clinically evident lipodystrophy in 8 of the 11 children after 4 years of therapy only. Although more objective measures for body composition and lipodystrophy are warranted, the rapid increase in weight-for-height *z* scores within 24 weeks makes an early development of lipodystrophy unlikely and suggests possible drug-related effects at a different level. Additional studies have to investigate whether an altered metabolism or energy expenditure may explain our finding in pediatric patients, as recently suggested by a study of PIs on protein catabolism[32,33] HIV infection may interfere with sexual maturation and the onset of puberty.[34] This could influence especially the growth velocity. However, in our cohort, the median age at start of HAART was 4.7 years (IQR 1.1 - 8.8); leaving out the oldest quartile from the analysis, similar growth parameters were obtained (data not shown). Although the contribution of d4T to the development of lipodystrophy is not yet clearly proved, we have to consider that the combined use of d4T and NFV may have played an important role in the high prevalence of lipodystrophy in our cohort.

HIV itself and endocrinologic and immunologic factors in combination with social environment all may contribute to the growth-related phenomenon.[31,34-36] No relevant alteration in endocrinologic parameters was found in prior studies.[35]

Protease-containing regimens have demonstrated a more profound effect on growth, especially in children who reached undetectable pVL and in those with advanced disease at baseline.[37-40] Growth was independent of virologic success in our cohort.

Conclusions

We have demonstrated that an NFV-based HAART regimen can be given safely over a long period of almost 7 years. Although the criteria of when to start HAART have changed over time[15,16], the clinical implications of our findings on a strong association between young age and virologic failure are important. In the light of our data and recent discussion on clinical practice and regimen switches[41,42], when to start with HAART in young children remains unclear and may be reconsidered.

Given the high virologic failure rate at young age observed in our cohort and the rather high prevalence of lipodystrophy, one should address questions about adherence, long-term exposure to HAART, and adverse effects when considering early initiation of HAART in children. Once treatment has been decided upon, it needs to be investigated whether there is a role for directly observed therapy to improve and guarantee both adherence and virologic success.

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3 Once-daily HAART in HIV-infected children: safety and efficacy of an efavirenz-containing regimen

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Summary

Background In order to improve compliance and virologic suppression we assessed the feasibility and effectiveness of a once-daily regimen of efavirenz with three nucleoside reverse transcriptase inhibitors (NRTIs) as 1st- and 2nd-line HAART in a cohort of HIV-1-infected children.

Methods: HIV-1-infected children naive for efavirenz were treated with a combination of efavirenz with abacavir, didanosine (ddI), and lamivudine (3TC) as 1st- or 2nd-line HAART in an observational, prospective, single-center study. Virologic failure-free survival was assessed by Kaplan-Meier analysis. The increase in $CD4^+$ T cells was estimated with a generalized linear model incorporating repeated measurements.

Findings Thirty-six children were on study medication for a median of 66 weeks (IQR: 39-118 weeks). Virologic failure-free survival rates were 76% and 67% after 48 weeks and 96 weeks respectively. No significant difference was found in efficacy between 1st- and 2nd-line HAART (p=0.7). All children on HAART showed a sustained $CD4^+$ T cell increase, irrespective of virologic suppression. Growth rates improved under HAART. In 14 children study medication was stopped, mostly because of non-adherence (4) or virologic rebound (5) and in 2 patients because of adverse events (unrelated death, grade-2 liver toxicity). Lipid abnormalities or abacavir-related hypersensitivity reactions were not observed.

Interpretation For the first time in HIV-1 infected children, once-daily HAART is demonstrated to be a safe, convenient and potent antiretroviral regimen.

Introduction

Since HAART became the standard of treatment for HIV-1-infected children, morbidity and mortality have declined significantly [1,2]. However, with the long-term use of HAART the limitations are becoming apparent.

Recommended as initial therapy are the combination of two nucleoside analog reverse-transcriptase inhibitors (NRTI) with either one protease inhibitor (PI), or one non-nucleoside reverse transcriptase inhibitor (NNRTI) [3,4]. Regarding effectiveness, any PI-containing first-line HAART in children seems efficacious after 2 years in 42 to 87% of the children [5-7].

A PI-containing regimen may have the potential for development of blood lipid disturbances and lipodystrophy, as we and others have observed [8-10]. The disfiguring appearance of lipodystrophy can also negatively influence the patients' compliance to HAART. Alternatively, PI-sparing regimens in adults using efavirenz combined with two NRTIs showed a virologic response of 70% of treated individuals having HIV RNA < 400 copies/mL at 48 weeks [11]. In HIV-1 infected children, it was shown that substitution of a PI by efavirenz resulted in the maintenance of virologic control in 17 children in whom HIV-1 was well suppressed [12]. A positive effect on the lipid profile was seen in this patient population. Therefore, efavirenz appears to be a suitable alternative to PIs. However, data regarding its use in once-daily regimens in children has not been described before.

A meta-analysis of virologic outcome data from clinical trials of various HAART regimens found a significant correlation between lower pill burden and treatment efficacy in adult patients [13]. In a pediatric population, compliance can be additionally compromised due to the patient's young age, poor palatability of the medications, and dependence on their caregivers. A once-daily regimen was therefore preferred.

According to the history of antiretroviral therapy, some children in our cohort had initially been treated with zidovudine, followed by zidovudine combined with didanosine (ddI) or zalcitabine (ddC), until the introduction of nelfinavir as the first PI available for children. Considering the high plasma HIV-1 RNA load (pVL) observed in young children compared to those in adults [14-16], a robust regimen was assumed to be required to avoid the early occurrence of new mutations in the viral reverse-transcriptase (RT) gene associated with resistance against antiretroviral drugs [17]. A duo-class regimen with four drugs containing abacavir was reported to be successful [18,19]. Thus, to increase compliance and virologic success rates we commenced a once-daily HAART regimen containing efavirenz and 3 NRTIs (i.e. abacavir, ddI and 3TC), and describe its safety, tolerability and effectiveness in HIV-1 infected children for up to 2 years.

Methods

Patients

Between January 2002 and August 2005 a prospective, observational study was performed. HIV-1-infected children were eligible, when they were aged 3 months to 18 years, and had a $CD4^+$ T cell counts < 1750/μL for those who were younger than 1 year, < 1000/μL for those who were between 1 and 2 years, < 750/μL for those who were between 3 and 6 years, and < 500/μL for those who were older than 6 years. Prior exposure to antiretroviral regimens was allowed. Exclusion-criteria consisted of the presence of resistance-associated mutations to efavirenz or to two or more of the NRTI study drugs used upon the commencement of the once-daily treatment regimen, pregnancy and HLA-typing unfavorable with respect to abacavir use [20]. No restrictions were made with regard to ethnicity, gender, route of HIV acquisition or disease stage. The Medical Ethical Committee of our institute approved the protocol. Parents or caregivers gave written informed consent.

Medication

Patients received efavirenz, abacavir, ddI and 3TC. Dosage adjustments were performed according to the weight of the children and, in case of efavirenz, consecutive plasma levels (to establish a trough level above 1 mg/L, which is considered a target value for virologic success in adults [21] and children [Crommentuijn, Scherpbier, Huitema, Kuijpers, Beijnen; in preparation]). It was recommended that the children take their regimen with food. When taken as solution for the optimal treatment of small children in our cohort, ddI was prepared with the acid-binding magnesium hydroxide, according to the prescription of the manufacturer.

Compliance

The children's guardians were counseled on the importance of treatment compliance. Where appropriate, the children were also counseled accordingly. Members of the treatment team monitored compliance by telephoning the guardians soon after the regimen was started and at each follow-up clinic visit.

Procedures

At each visit physical examination was performed including weight, length and head circumference measurements. Blood was drawn prior to, and at 1 and 2 weeks and 1, 2 and 3 months after initiation of HAART, and every 3 to 4 months thereafter. Lymphocyte subsets were analyzed using FACScan (Becton Dickinson, San Jose, CA, USA). Plasma viral load (pVL) was measured using Versant HIV-1 bDNA 3.0 (Bayer, Mijdrecht, the Netherlands) with a LLQ of 50 copies/mL (input 1 mL of plasma). Virologic failure was defined as two consecutive pVL > 50 copies/mL. Patients who never reached a pVL < 50 copies/mL, were failing at the 1st measurement that was higher than the previous one after initial decline in pVL.

Nucleotide sequence analysis of the HIV-1 protease and RT genes was performed at baseline and upon virologic failure. Sequence analyses were performed using the Viroseq

HIV-1 genotyping kit version 2 (Abbott laboratories, IL, USA). Resistance conferring mutations were screened as described by the International AIDS Society-USA [www.iasusa.org].

Adverse events were recorded during the study period and defined as any clinical sign or symptom, or meaningful laboratory test abnormality, possibly or probably related to the study medication, excluding HIV-related disorders. The National Institute of Allergy and Infectious Diseases (NIAID / Division of AIDS) toxicity table was used for grading severity of pediatric adverse experiences. Parents were asked for the presence of side effects at every visit.

Statistical analysis

The primary outcome was virologic failure-free survival, which was assessed using Kaplan-Meier analysis. Censoring was applied if the last patient visit or a switch to another regimen occurred before virologic failure. The secondary outcome were factors associated with virologic failure, changes in $CD4^+$ and $CD8^+$ T cells over time, changes in growth parameters (weight, height) over time, reported adverse events and the occurrence of resistance mutations. Age-adjusted $CD4^+$ and $CD8^+$ T cell ratios were calculated by dividing the counts by the mean of an age-matched healthy control group [22]. Growth of the children was analyzed by means of the z scores (standard normal deviation) of height and length. These scores were calculated with the use of the Growth Analyser 2.0 software (Dutch Growth Foundation, Rotterdam, the Netherlands) using Dutch reference values. Age-adjusted $CD4^+$ and $CD8^+$ T cell ratios and height and weight z scores were modeled using a mixed model incorporating repeated measurements. This model handles missing data adequately by estimating the outcome given a specific covariate structure. The estimates of a specific level of the fixed effects were modeled using the 'first order autoregressive' approach. Differences in these estimates between different levels of the variable were tested for significance using the t-statistic. Where subgroups of patients are compared, the differences between groups were evaluated using the Fisher's exact test for categorical data and the Kruskal Wallis test for continuous data. All statistical analyses were performed using SPSS for Windows version 11.5 (SPSS Chicago). A two-sided p-value < 0.05 was considered statistically significant.

Results

Patients

All 36 HIV-1-infected children who started a once daily antiretroviral regimen with efavirenz between January 2002 and August 2005 were included in the present analyses. Antiretroviral-naive, as well as pretreated HIV-1-infected children were included. Twenty-two children (61%) had been on HAART for a median 259 weeks prior to enrolment (Interquartile range (IQR) 104 – 310 weeks). Of these children 10 were also pre-treated with mono/duo NRTI therapy for a median of 134 weeks prior to the start with HAART.

Baseline characteristics are shown in Table 1. The median age of the children at baseline was 6.6 years (IQR: 3.3 - 10.7 years). One of the children was younger than 1 year, 6 were between 1 and 2 years, 12 were between 3 and 6 years, and 17 were older than 6 years. Children naive to antiretroviral therapy were younger at baseline than children that received 2nd-line HAART (median 3.3 years (IQR: 1.7 - 9.9) vs 8.8 years (IQR: 5.2 - 11.5), p=0.04). Thirty-four children (94%) acquired HIV infection perinatally from their HIV-1-infected mother, 15 (42%) children presented with CDC-C classified AIDS defining symptoms. The majority of the children were black (African or Surinamese). The children were on study medication for a median duration of 69 weeks (IQR 39 – 122 weeks).

Virology

At baseline, the median pVL for the whole group was 3.6 log copies/mL (IQR 2.4 - 4.7). Children that started the once-daily regimen as 2nd-line HAART had a significantly lower pVL than children that started antiretroviral naive with the regimen (median 2.5 vs. 5.4 log copies/mL, p<0.001). Twelve of 22 (55%) children that started 2nd-line HAART were undetectable at switch of therapy. The virologic failure-free survival rates were 76% and 67%, after 48 and 96 weeks, respectively (Fig 1A). Twelve children completed a follow-up of 96 weeks on study medication. Of the patients who failed on therapy or discontinued

TABLE 1 Baseline characteristics of children starting with the efavirenz-containing study regimen and comparison between 1st and 2nd line HAART

	Total	1st line HAART	2nd line HAART
Number of patients	36	14	22
Female	19		
Age, yrs[1]	6.6 (3.3-10.7)	3.3 (1.7-9.9)	8.8 (5.2-11.5)
CDC- C[2]	15		
Vertical	34		
Sexual	3		
Black	32		
non-black	4		
CD4+ T cells, abs per µL[1]	730 (400-1050)	460 (170-940)	860 (600-1170)
CD4+ T cells, %[1]	26 (16-37)	14 (6-25)	31 (25-38)
CD4+ T cells, age adjusted[1]	0.5 (0.3-0.9)	0.3 (0.1-0.5)	0.7 (0.4-0.9)
CD8+ T cells, abs per µL[1]	1270 (800-1910)	2060 (790-3480)	1120 (810-1400)
CD8+ T cells, %[1]	44 (34-61)	58 (38-72)	38 (31-49)
CD8+ T cells, age adjusted[1]	1.5 (1.1-1.8)	1.9 (1.2-2.9)	1.4 (1.0-1.6)
Total cholesterol, mmol/L	3.9 (3.4-4.5)	3.4 (3.1-3.6)	4.3 (3.9-4.7)
Triglicerides, mmol/L	0.8 (0.6-1.5)	1.2 (0.8-1.7)	0.7 (0.6-1.0)
HIV-1-RNA, log copies/mL[1]	3.6 (2.4-4.7)	5.4 (4.4-6.0)	2.5 (2.4-3.5)
Height–for–age[1]	–1.2 (–2.0- –0.1)	–1.9 (–3.0- –1.3)	–0.5 (–1.4- 0.5)
Weight–for–height[1]	0.6 (–0.5-1.2)	0.6 (–0.5-1.5)	0.5 (–0.5-1.0)

[1] median, interquartiles between brackets (IQR), [2] Clinical categories as defined by the US Centers for Disease Control and Prevention (34).

study medication at any time during the follow-up, 6 never had a pVL below the LLQ. Of the remaining 30 children in this observational cohort, 4 showed a rebound of their pVL after having had a period of viral suppression below the LLQ after the initiation of study medication. The effect of prior HAART on virologic effectiveness was analyzed with a log rank test; there was no difference in virologic responders and non-responders (p = 0.7) (Fig 1B).

Reasons for treatment discontinuation

Study medication had to be discontinued in 14 (39%) children during follow-up for the following reasons: 5 virologic failures with several new mutations, 4 reported non-compliances, 2 due to aversion to taste of the medication, 1 pregnancy, 1 serious adverse event (death) and 1 adverse event (grade-2 elevation in liver transaminases). The fatality

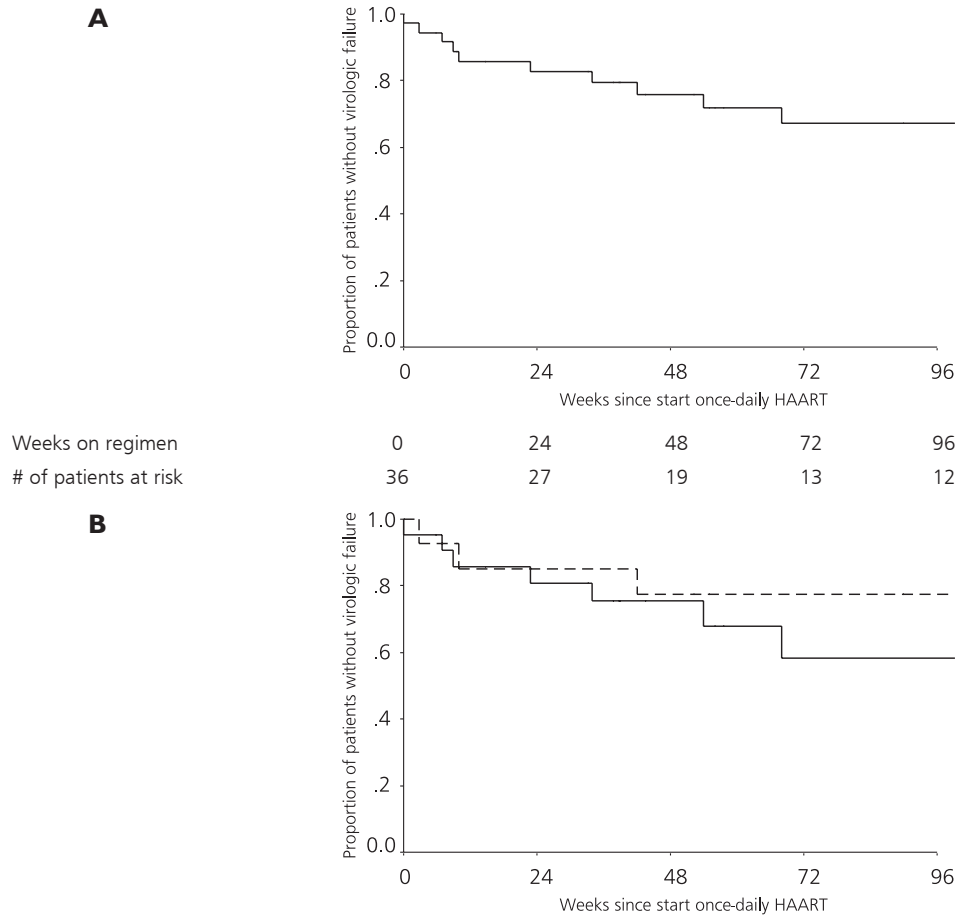

FIGURE 1 A. Kaplan-Meier survival analysis of time to virologic failure. Numbers of patients at risk at start and after 1 year are indicated. Censoring was applied if the last patient visit or a switch to a simplified regimen occurred before virologic failure.

FIGURE 1 B. No difference in virologic responders and non-responders was observed when children on 1st-line (dotted line) and children on 2nd-line HAART (straight line) were analyzed in a multivariate Cox proportional hazards model (p=0.7).

TABLE 2 Resistance mutations at baseline and after failure

#	Age[1]	Naive[2]	HAART [3]	Resistance mutations for RT [4]	
Failures				Prior to study medication	On study medication
1	2.6*	**N**		–	**181C, 230L**
2	18.3*	E	ABC > TDF	179E	**103N, 106A, 65R**
3	14.9*	E		–	–
4	4.9*	E		70R, 184V	**103N**
5	9.3**	E		184V	**103N, 108I**
6	4.4*	E		67N, 69N/D/A, 70R, 98S, 184V, 219Q	**190E**
7	0.9*	**N**		–	**103N, 225H**
8	5.3**	E		41L, 98S, 184V, 215Y	**103N, 188L, 74V**
9	13.5**	E		70R, 184V	–
10	5.2**	**N**		–	**106M,** 65R, 75I, 115F
Responders					
1	14.6	E		67N, 69N, 70R, **181C**, 184V	
2	1.4	**N**		–	
3	10.8	E		184V, 210W, 215Y	
4	5.9	E		67N, 70R, 184V, 179I, 219Q	
5	16.5	**N**		–	74V, 184V
6	3.7	E		184V	
7	2.2	E		184V	
8	3.4	**N**		–	
9	9.2	E		–	
10	10.4	E	ABC > ATV/r	41L, 44D, 67N, 69D, 184V, 215Y	
11	9.7	E		–	
12	6.6	**N**		–	
13	18.9	E	ABC > TDF; ddI > LPV/r	41L, 44A, 62V, 118I, 184V, 210W, 215Y	
14	8.5	E		67N, 70R, 101Q, 179I, 184V, 219Q	
15	3.1	E		184V	
16	10.9	**N**		–	
17	15.1	**N**		–	
18	9.5	**N**		–	
19	1.8	**N**		–	
20	6.5	E		184V	
21	2.6	**N**		**69N**	
22	10.4	E		41L, 62V, 184V, 210W, 215Y	
23	7.9	E		–	
24	1.2	**N**		–	
25	3.3	**N**		–	
26	6.7	E		–	

[1] age in years, [2] naive to treatment (N) or treatment experienced (E), [3] HAART study medication consisted of ABC ddI, 3TC, and EFV; in some cases study medication was adapted using tenofovir (TDF), or ritonavir-boosted lopinavir (LPV/r) or atazanavir (ATV/r), [4] resistance mutations against the viral reverse transcriptase (RT) scored according to the International AIDS Society-USA [www.iasusa.org]. * Non-responder without viral suppression <50 copies/mL upon start of study medication, ** Rebound of pVL after viral suppression <50 copies/mL upon start of study medication.

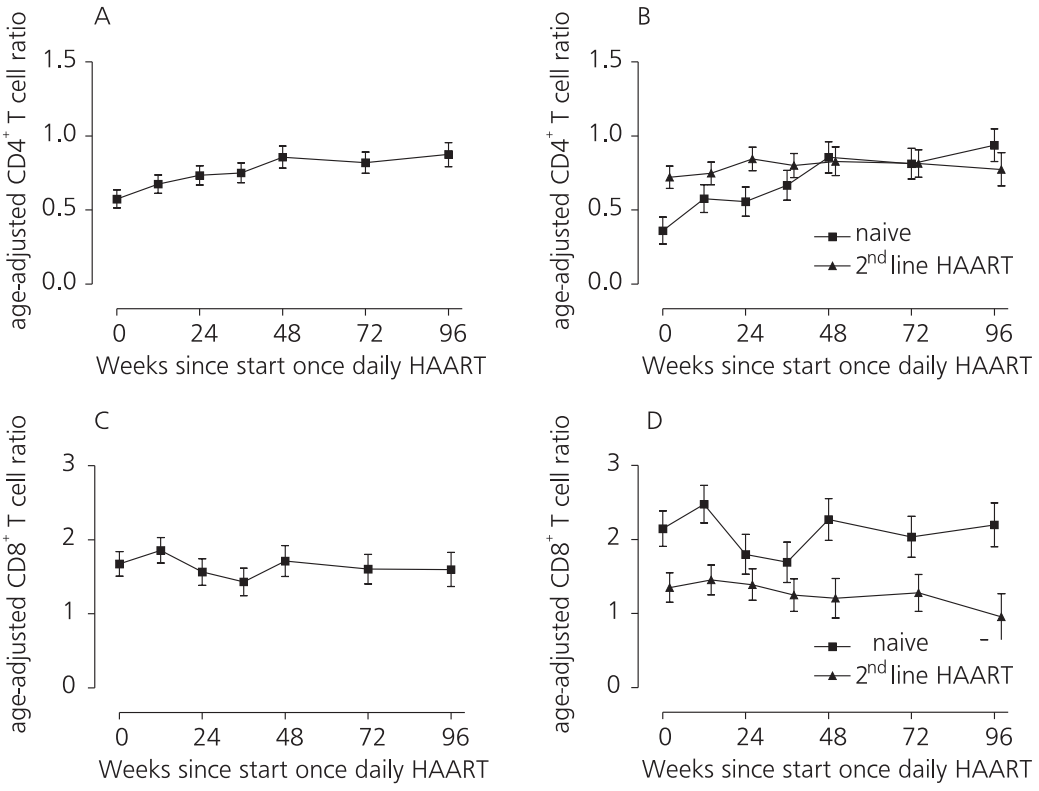

FIGURE 2 A. Age-adjusted CD4$^+$ T-cell count during 96 weeks follow-up on HAART. Follow-up of all patients during treatment with the study medication. **B.** A comparison of children on 1st and 2nd line HAART. **C.** Age-adjusted CD8$^+$ T-cell count during 96 weeks follow-up on HAART. Follow-up of all patients during treatment with the study medication. **D.** A comparison of children on 1st and 2nd line HAART. Bars indicate standard errors of the mean.

occurred in a patient who experienced a severe electrolyte disturbance and lactate acidosis due to persistent diarrhea despite rapid virologic response to undetectable levels. In the opinion of the treating physicians this was not attributable to the study drugs. Hypersensitivity to abacavir was not seen.

Resistance mutations

The RT gene from HIV-1 in plasma samples was sequenced from all 36 children. The HIV-1 strains in children failing to the study medication were scrutinized for the occurrence of additional critical mutations in the RT gene associated with NNRTI resistance, efavirenz in particular (i.e., 100I, 103N, 106A/M, 108I, 181C/I, 190A/S, 225H, 230L). One HAART-experienced boy had a 181C mutation at the start of the study regimen. His pVL became undetectable under study medication. In one child naive to antiretroviral drugs a 69N mutation in RT was found at baseline. Mutations associated with resistance to one or more NRTIs were detected in the group of children that had previously shown viral blips or had completely failed on their 1st-line PI-containing HAART regimen (Table 2).

In a survival analysis, there was no significant difference in time to virologic failure in the patients with existing mutations at baseline compared to children without mutations at baseline (p=0.5).

Immunology

At baseline, the median $CD4^+$ T cell count for the total study population was 730/μL (IQR: 400 - 1050) and age-adjusted $CD4^+$ T cell ratio 0.5 (IQR: 0.3 - 0.9), the $CD4^+$ T cell percentage was 26% (IQR: 16 - 37). The baseline age-adjusted $CD4^+$ T cell ratio, absolute number, and percentage of $CD4^+$ T cells were statistically significantly higher in children who started the regimen as 2nd-line HAART, compared to children who had not received previous antiretroviral medication (p=0.003, p=0.03, p<0.001, respectively) (Table 1). The median $CD8^+$ T cell counts for the total study population was 1270/μL (IQR: 800 - 1910) and age-adjusted $CD8^+$ T cell ratio was 1.5 (1.1 - 1.8).

The median age-adjusted $CD4^+$ T cell ratio demonstrated an increase during the 96 weeks on treatment (Fig 2A). Children that started naive to antiretroviral therapy had a more profound increase compared to children on 2nd-line HAART (Fig 2B). This was due to a lower baseline $CD4^+$ T cell count. The age-adjusted $CD8^+$ T cell ratios demonstrated a slight but non-significant decrease in the total study population (Fig 2C) as well as in both subgroups based on pretreatment (Fig 2D).

Lipids

Although there was a significantly lower total cholesterol in patients that started naive to antiretroviral therapy than in patients that started 2nd-line HAART (median 3.4 vs. 4.3, p<0.001) all children were below the cut-off of 6.5 mmol/L (upper limit of the normal range). The same applied for triglycerides at baseline (median 1.1 vs.0.7 mmol/L, p=0.04; normal levels <5.0 mmol/L).

During the treatment with HAART total cholesterol increased. However, in children with 2nd-line HAART total cholesterol remained stable. Children that started naive to antiretroviral therapy showed an increase towards the values of the group with 2nd-line HAART within the first weeks. Triglycerides did not change over time during treatment with the once-daily regimen.

Growth and development

Growth parameters are shown in Figure 3. The median height-for-age z-score at baseline for the total study population was –1.2, and the median weight-for-height z-score was 0.6. Children naive to antiretroviral therapy had a significantly lower height-for-age z-score than children on 2nd-line HAART (median z-score –1.9 vs. –0.5, p=0.001). The naive group showed a distinct increase in the first 48 weeks but did not reach the level of the 2nd line HAART group (Fig 3B). An increase in weight-for-age z-score was seen during 96 weeks on treatment to almost normal (Fig 3C). Children with 2nd-line HAART showed a different pattern over time than children that started naive to antiretroviral drugs (Fig 3D). The children that started naive to antiretroviral therapy showed an increase in contrast to the children on 2nd-line HAART, showing a higher baseline that remained stable. Weight-for-height z-scores remained stable in both treatment groups (Fig 3E & F).

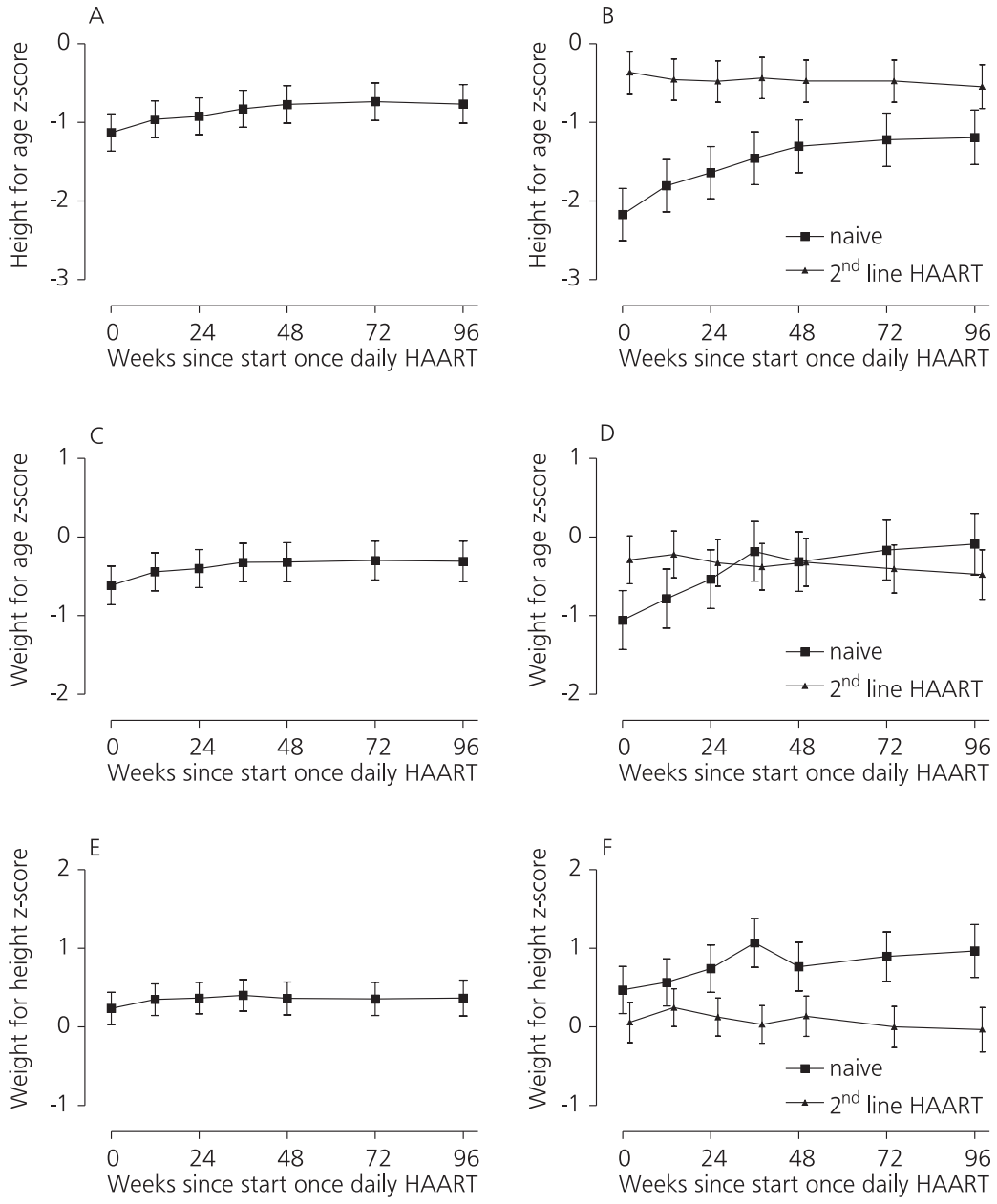

FIGURE 3 A. Height-for-age z scores during 96 weeks follow-up on HAART. **B.** A comparison of children on 1st and 2nd line HAART. **C.** Weight-for-age z scores during 96 weeks follow-up on HAART. **D.** A comparison of children on 1st and 2nd line HAART. **E.** Weight-for-height z scores during 96 weeks follow-up on HAART. **F.** A comparison of children on 1st and 2nd line HAART. Follow-up of all patients during treatment with the study medication. Z scores were calculated for each measurement of height according to age and gender using the 1997 Dutch reference curves. Bars indicate standard errors of the mean.

Discussion

We demonstrated for the first time virologic effectiveness, tolerability and safety of a once-daily HAART regimen in an HIV-1-infected pediatric cohort. Virologic failure-free survival rates were 76% and 67% after 48 and 96 weeks respectively for all children with equal effectiveness of the study regimen when used as 1st- or 2nd-line HAART (77% vs. 75% after 48 weeks). To date, the only pediatric report on efavirenz-containing HAART is in 17 children with persistently suppressed pVL switching to efavirenz-containing HAART for reasons of convenience and simplification [12]. Our treatment regimen contained efavirenz and three NRTIs as the backbone and was administered to 36 children. A limited number of studies on once-daily regimens in treatment-naive adults have been reported up to 48 weeks, ranging from 50-78% virologic failure-free survival [23-28]. The combination of tenofovir-ddI as the NRTI backbone seemed the least effective [26,27], despite good adherence as defined by Medication Event Monitoring System (MEMScap) and plasma EFV concentration monitoring [27]. Our results in children favorably demonstrate the strong antiretroviral activity of the chosen once-daily regimen (irrespective of prior treatment-experience).

Our efavirenz-containing once-daily regimen was well tolerated. Regarding safety and tolerability, we observed medication-related grade-2 toxicity in only one child consisting of a rise in blood liver enzymes. This is in concurrence with other reports in children [12,15,29]. The non-fasting lipids remained within normal ranges, although total cholesterol showed an increase during the first weeks in the naive group compared to the children that started 2nd-line HAART. In the 2NN study it was shown that the efavirenz-associated increase in total cholesterol was mainly due to HDL cholesterol [30]. One case with AIDS-related severe cachexia died due to persisting electrolyte disturbances in spite of very successful HIV-1 suppression. In 14 children the regimen was stopped despite good tolerability and simplification of intake compared to most of the previous regimens in children. Discontinuation had several reasons but mostly for reasons of virologic rebound due to assumed or self-reported non-compliance.

A latent viral reservoir may harbor viruses that are generated at various times throughout the life of perinatally infected children, including wild-type, drug-sensitive viruses transmitted from the mother and any drug-resistant viruses arising during nonsuppressive, (pre) HAART therapy [17]. Seventeen of the 22 receiving 2nd-line HAART showed extensive RT mutations. In one child naive to antiretroviral drugs a 69N mutation in RT was found at baseline. This mutation is associated with resistance to zidovudine, d4T and ddI. Most probably the virus was acquired from the mother, although the predominant virus population in the mother appeared to contain a 69S mutation in RT. The difference in amino acid at this position can be explained by viral evolution since the baseline sequences of mother and child were obtained 3.5 years after birth. One HAART-experienced boy had a 181C mutation at the start of the study regimen. Although this mutation is associated with resistance to efavirenz, it has greater impact on the sensitivity

to nevirapine [31]. With the addition of lopinavir/ritonavir to the study regimen a lasting virologic response was achieved.

Apart from pre-existing mutations, the impact of adherence on the effectiveness of HAART must be seriously considered. Of the virologic failures, 2 patients previously treated with HAART without mutations reported non-adherence to the regimen by themselves. In most of the other virologic failures, efavirenz was repeatedly below the trough level of 1 mg/L even after dose adjustments, and hence suspected of non-compliance. In line herewith, critical NNRTI-associated resistance mutations were found in 7 of these 8 patients. Similar to Luzuriaga et al [32], we did not observe an association between pre-existing NRTI (or PI)-resistance mutations and success or failure of virologic control (p=0.5), suggesting that adherence may indeed be the most important factor of lasting virologic suppression in our cohort.

The increase in $CD4^+$ T cells was observed in both groups, although the rise in cell number was more profound in the group that started naive to antiretroviral therapy due to the lower baseline counts. As expected, the baseline age-adjusted $CD4^+$ T cell ratio and absolute and relative $CD4^+$ T cell counts were statistically significantly higher in children who started the regimen as 2^{nd}-line HAART. No severe clinical infections occurred during the study period in either group, irrespective of virologic failure.

With respect to general growth and development, naive children showed an increase in height-for-age z-scores but did not reach the level of the 2^{nd}-line HAART group at 96 weeks. In our cohort the children on 1^{st}-line HAART showed normalization of weight-for-age z-scores whereas the 2^{nd}-line HAART group already had almost normal z-scores at start. Nachman et al described similar findings in 192 clinically stable children, of which 50% were pretreated with NRTIs but naive to the new HAART regimens [33]. Most of the 2^{nd}-line HAART group in our study had used stavudine, lamivudine and nelfinavir as 1^{st}-line HAART for a median of 259 weeks. Almost 30% had developed clinically evident lipodystrophy [10].

The follow-up in the present once-daily study is too short to expect any effect on fat distribution. Moreover, the number of naive children is small and total treatment follow-up is relatively short to judge for any alteration in this respect.

In conclusion, this is the first study of a once-daily efavirenz containing HAART regimen in a pediatric cohort. Our study demonstrates virologic and immunologic effectiveness, even in children that were HAART-experienced. We can conclude that the once-daily regimen used is a convenient, safe and robust regimen for children. Most children were antiretroviral drug-experienced but demonstrated equal effectiveness of the once-daily regimen compared to children on 1^{st}-line HAART. Because of the high pVL in young children [14-16] a robust regimen is required. A duo-class regimen with four drugs containing abacavir was considered more successful [18,19]. Simplification of the study regimen by stopping one of the NRTIs when the pVL is below the LLQ may be a possibility with similar outcome and efficacy. A considerable number of patients that stopped the treatment regimen admitted non-adherence or were suspected of incompliance

because of low drug levels in consecutive blood samples, irrespective of the dose adjustments made. Further improvement of effectiveness of treatment can be reached in the future since once-daily regimens allow for various sorts of measures to support adherence (such as MEMScap or directly observed therapy), in larger well-designed studies on virologic outcome.

Acknowledgments

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4 Immune reconstitution in HIV-1 infected children treated with HAART is independent of their age and pretreatment immune status

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AIDS 2001;15:2267-75

Abstract

Objective

To evaluate long-term immune reconstitution of children treated with highly active antiretroviral therapy (HAART).

Methods

The long-term immunological response to HAART was studied in 71 HIV-1-infected children (age: 1 month to 18 years).in 2 prospective, open, uncontrolled national multicentre studies.
Blood samples were taken before and after HAART was initiated with a follow-up of 96 weeks, and peripheral CD4+ T-cells, CD8+ T-cells, naive and memory subsets were identified on whole blood samples. Relative cell counts were calculated in relation to the median of the age-specific reference.

Results

The absolute CD4+ T-cell count, and percentage and the CD4+ T-cell count as a percentage of normal increased significantly ($p<0.001$) to medians of 939 x 10^6 cells/L (range 10-3520), 32% (range 1-50) and 84% (range 1-161) respectively after 48 weeks. This increase was predominantly naive CD4+ T-cells. There was a correlation between the increase of absolute naive CD4+ T-cell counts and age. However, when CD4+ T-cell restoration was studied as percentage of normal values, the inverse correlation between the increase of naive CD4+ T-cell count and age was not observed. In addition, no difference in immunologic reconstitution was observed at any timepoint between virologic responders and non-responders.

Conclusions

Normalization of the CD4+ T-cell counts in children treated with HAART is independent of age, indicating that children of all age-groups can meet their CD4+ T cell production demands. In general, it appears that children restore their CD4+ T-cell counts better and more rapidly than adults, even in a late stage of HIV-1 infection.

Introduction

Highly active antiretroviral therapy (HAART) has only recently been applied in children infected with HIV-1. The use of HAART leads to a reduction in plasma HIV-1 RNA loads to undetectable levels in a high percentage of these children and results in a significant recovery of CD4+ lymphocyte counts. (1-6)

In adults immune reconstitution following HAART shows a biphasic pattern consisting of an initial rapid redistribution of memory cells and a gradual slow increase in naive T-cells. (7, 8) Children with HIV-1 infection have a greater capacity to reconstitute their naive CD4+ T-cells compared with HIV-1-infected adults treated with similar antiretroviral therapy. (6) This is not an unexpected finding, since naive T-cell recovery is believed to be thymus-dependent and thymic function diminishes with age. (9, 10) Increased production of naive cells is associated with thymic enlargement in children treated with anti-cancer chemotherapy as well as in children treated with HAART as shown on radiographs or by magnetic resonance imaging. (9, 11, 12)

CD4+ T-cell numbers in HIV-1-infected children on HAART recover more rapidly than CD4+ T-cells in HIV-1-infected adults. (6, 11, 13-16) However, it is still unclear to what extent the number of CD4+ T-cells of HIV-1-infected children is capable of returning to normal levels, since data on long-term T-cell dynamics in HIV-1-infected children on HAART are not available. In a considerable number of HIV-1- infected adults treated with HAART, CD4+ T-cell numbers stabilised or even slightly decreased after 18 months of therapy, sometimes without having reached normal levels. (17) Long-term immune reconstitution is evaluated in HIV-1-infected children who were treated with HAART, consisting of one protease inhibitor and two nucleoside reverse transcriptase inhibitors during a period of 96 weeks. Changes in the number of CD4+ T-cells, CD4+ T-cell naive and memory subsets and CD8+ T-cell counts were analysed and compared with the normal values that were previously reported for the different age groups. (18, 19)

In addition to the quantitative analyses, T-cell function was analysed after stimulation with monoclonal antibodies (mAb) for CD3 and for CD28 was analysed.

Methods

Patients

Seventy-one HIV-1-infected children were enrolled in two prospective, open, uncontrolled, studies to evaluate the clinical, immunological and virological response to combination therapy with either indinavir, zidovudine and lamivudine or nelfinavir, stavudine and lamivudine. Inclusion and exclusion criteria for these studies were equal and have previously been described in detail. (3) Children 1 month to 18 years of age, with or without prior treatment with reverse transcriptase inhibitors, and a viral load of more than 5000 copies/ml or a CD4+ T-cell count less than the lower limit of the age-

specific reference value were eligible for enrolment. Study protocols were approved by the medical ethical committee of all participating institutions. Written informed consent was obtained from the parents or the legal guardian. Blood samples were taken before HAART was started (2 to 0 weeks prior to initiation of the study) and at 1, 2, 4, 8, 12, 24, 36, 48 and 96 weeks after the initiation of combination therapy. Medication was given in the following doses: indinavir 400 mg/m^2 every 8h, nelfinavir 30 mg/kg every 8h, zidovudine 120 mg/m^2 every 8h, lamivudine 4 mg/kg every 12h and stavudine 1 mg/kg every 12h. Multiple dose pharmacokinetics of either indinavir of nelfinavir were determined four weeks after HAART was initiated. A dosage adjustment of indinavir or nelfinavir was done when necessary to adjust the area under the time concentration (AUC) curve to adult values.

Immunophenotyping and T-cell function *in vitro*

Lymphocyte immunophenotyping of peripheral CD4+ and CD8+ T cells was performed on lysed whole blood samples by flow cytometry using triple staining. Lymphocytes were phenotyped as naive and memory CD4+ and CD8+ T-cells by three-color immunofluorescence flow cytometry using combined staining with CD45RA and CD 62L (L-selectin) or CD27 monoclonal antibodies (mAb). T cells expressing both CD45RA and CD62L or CD27 were considered truly naive cells, whereas cells, that lacked either CD45RA and CD62L or CD27 were regarded as memory cells. (20, 21) Previously, it has been demonstrated that naive and memory subset distribution as measured with either CD45RA and CD62L mAb or CD45RA and CD27 mAb yielded identical results. (22) T-cell function was determined in whole-blood lymphocyte culture (23). Proliferative responses to CD3 mAb plus CD28 mAb were measured after 4 days of culture by means of the incorporation of ^{3}H-thymidine added 24 hours before harvest. (24) Proliferative capacity was calculated as counts per minute (cpm) per 10^3 CD3+ T-cells and results are expressed as percentage of the median of 3450 healthy adult donors (24).

Plama HIV-1 RNA determination

Plasma HIV-1 RNA levels were measured by an in vitro nucleic acid amplification test (Amplicor HIV-1 Monitor Test (Roche Diagnostic Systems, Branchberg, USA) with a lower limit of quantification of 500 copies/ml, by the NASBA assay (Nuclisens HIV-1 RNA; Organon Teknika, Boxtel, The Netherlands) with a lower limit of quantification of 400 copies/ml or by the Quantiplex b DNA test (Bayer, Mijdrecht, The Netherlands) with a lower limit of quantification of 50 copies/ml. The test used at baseline was also used at every follow-up visit. The on-treatment-analysis method was used to calculate percentages of patients with an HIV-1 RNA below the lower limit of quantification (LLQ) of 500 copies/ml.

Statistical analysis

All patients with analyses made at baseline (in 10 patients in which baseline values were missing, analyses at week 1 were considered as baseline) and at least 12 weeks of follow-up were included. SPSS 9.0 (SPSS, Chicago, Illinois, USA) was used for statistical analysis. Because absolute CD4+ T-cell counts are highly dependent on the age of the patients and on the stage of disease, relative CD4+ T-cell counts (total CD4+ T-cells and

naive CD4+ T-cell counts) in relation to the median of the age-specific reference values (18, 19) were calculated by dividing the individual value at the different time-points by the median of the reference value at the different time-points. Results are expressed as percentage of normal. Since CD4+ T-cell percentage of total T-cells is influenced by changes in the number of CD8+ T-cells, calculation of CD4+ T-cell counts as percentage of normal, results in a parameter that is independent of CD8+ T-cell count. Correlation coefficients were obtained by Spearman rank correlation. Differences between paired variables were analysed with the Wilcoxon signed rank test and between groups with the Mann-Whitney *U* test. All p-values are two-tailed.

Results

Baseline characteristics of the 71 patients are presented in Table 1; 42 of 71 (59%) patients were not pre-treated and 29 (41%) had received prior treatment with nucleoside reverse transcriptase inhibitors (mostly with zidovudine monotherapy for an average of 32 months). Thirteen of the children with prior zidovudine therapy were placed on the stavudine arm, and the other 16 continued zidovudine. Baseline viral load and prior treatment did not significantly correlate with the plasma HIV-1 RNA at week 96. The change from baseline in absolute CD4+ T-cell count, CD4+ T-cell count as percentage of total T-cells and CD4+ T-cell count as percentage of normal was not significantly different between children with or without prior treatment at all time points. The 96 weeks of follow-up were completed by 37 children; of those who did not complete 96 weeks of follow-up, four were lost to follow-up, one child died and the other 29 entered the study-cohort at times shorter than 96 weeks before the analysis.

Plasma HIV-1 RNA

The medians and interquartile ranges (IQR) of the viral load after start of HAART and the percentages of patients with a viral load below the LLQ in responders and non-responders are depicted in Figure 1. Virologic responders were defined either as those who reached an undetectable viral load (<500 or <400 copies/ml) or as those who had a > 1.5 $\log_{10}$ reduction in viral load compared with baseline at week 12 after the initiation of HAART which was maintained during the follow-up period. Fifty-six patients qualified as responders and fifteen as non-responders. In the virological responders, HIV-1 RNA was below the LLQ in 79% of the children after 12 weeks and in 91% after 48 weeks. Of the 37 children who completed 96 weeks of follow-up, 31 of these children were classified as responders and six as non-responders.

Naive and memory CD4+ T-cell responses

The median absolute CD4+ T-lymphocyte counts at baseline varied widely among patients (Table 1). According to the CDC guidelines (25) 14 (20%) patients did not show immunosuppression (CD4+ T-cell count ≥25% of total T-cell count), 28 (39%) showed moderate immunosuppression (CD4+ T-cell count 15-24% of total T-cell count) and 29

TABLE 1 Baseline characteristics of the study patients

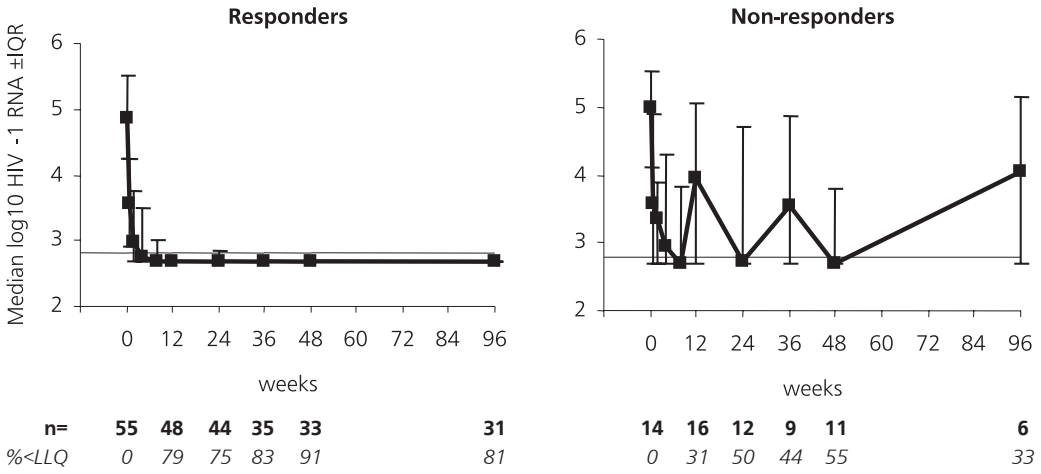
Characteristic				
Number of patients	71			
Age in years*	5.1 (0.1-17.5)			
Route of acquisition:				
Vertical	58			
Blood products	6			
Heterosexual	3			
Unknown	4			
Clinical stage (CDC-classification)†:				
N1 1	A1 6		B1 2	C1 3
N2 5	A2 13		B2 6	C2 12
N3 1	A3 3		B3 5	C3 14
HAART				
IDV/ZVD/3TC	32			
NFV/d4T/3TC	26			
NFV/ZDV/3TC	11			
NFV/d4T/ddI	2			
Median HIV RNA (copies/ml) *	82,000 (620-27,000,000)			
T -cells absolute (106 cells/L) *	All age groups	< 2 years	2-5 years	≥ 5 years
Number of patients	n = 71	n = 18	n = 17	n = 36
CD4+ T-cells	471 (0-3580)	767 (81-3580)	561 (2-1490)	370 (0-1140)
Naive‡ CD4+ T-cells	211 (0-2880)	967 (47-2880)	226 (0-1237)	167 (0-616)
Memory§ CD4+ T-cells	205 (1-2613)	570 (5-2613)	229 (2-426)	193 (1-646)
% of total T cells	17 (0-60)	22 (7-60)	17 (0-51)	17 (0-51)
% of normal	41 (0-143)	31 (3-143)	43 (0-115)	41 (0-114)
CD8+ T-cells	1147 (60-7369)	1680 (640-5436)	1070 (60-7360)	1030 (150-4860)
Naive CD8+ T-cells	257 (17-1312)	753 (363-1312)	246 (17-883)	188 (26-773)
Memory CD8+ T-cells	800 (24-6477)	734 (166-4131)	744 (24-6477)	816 (119-4228)
% of total T cells	44 (12-78)	34 (16-78)	36 (12-60)	52 (23-73)
% of normal	140 (7-920)	168 (64-494)	134 (7-920)	154 (19-744)

* Median (range)
† Clinical and immunological categories as defined by the US Centers for Disease Control and Prevention (CDC). (25)
‡Defined as CD45RA+/CD62L+ or CD45RA+/CD27mAb+ CD4+ T-cells
§ Defined as CD45RO+/CD62L- or CD45RO+/CD27mAb- CD4+ T-cells

(41%) showed severe immunosuppression (CD4+ T-cell count <15% of total T-cell count) at baseline.

The absolute CD4+ T-cell count and the CD4+ T-cell percentage both increased significantly ($p<0.001$) to a median of 939 x 10^6 cells/L (range 10-3520) and 32% (range 1-50%) respectively after 48 weeks of therapy (Figure 2A). In all age groups the increase of total absolute CD4+ T-cell counts was mainly caused by the increase of the naive CD4+ T-cell subpopulation. Memory CD4+ T-cells did not increase significantly. Consequently the increase of the CD4+ T-cell counts in children was not biphasic as in adults. After an initial increase of the total CD4+ T-cell counts and naive CD4+ T-cell counts, a

FIGURE 1 Medians and interquartile ranges (IQR) of HIV-1 RNA with a lower limit of quantification (LLQ) of 500 copies/ml in virologic responders and non-responders after the initiation of HAART. The number of patients which are analysed and the percentage of values below LLQ are indicated.



decrease was seen at week 8 in children younger than 5 years. Outliers could not explain this decrease. After 48 weeks CD4+ T-cell counts reach a plateau consisting of values equivalent to the CD4+ T-cell counts in healthy children. In children younger than 2 years absolute CD4+ T-cell counts even decreased after 48 weeks, reflecting the normal age-related strong decline of CD4+ T-cell counts in children at this age.

The increase of the CD4+ T-cell percentage in the three age groups is depicted in Figure 2B. The ratio of naiveto memory CD4+ T-cell counts for the total group increased significantly (p=0.003) from 0.67 (range 0.04-4.88) to 1.70 (range 0.09-6.69) after 48 weeks, reflecting the increase mainly consisting of naive CD4+ T-cells.

CD4+ T-cell counts expressed as percentage of normal, increased significantly (p<0.001) from 44% (range 0-143%) to 84% (range 1-161%) after 48 weeks. The change of CD4+ T-cell count from baseline was not significantly different between the age groups at any time-point.

CD4+ T-cell response and age

High CD4+ T-cell recovery rates have been associated with younger age. (6, 11, 13-16) Indeed a tendency to an inverse correlation was found between the increase of absolute naive CD4+ T-cell counts and the age of the children after 4, 24 (Figure 3A) 36 and 48 weeks of HAART (r= -0.31, p= .03; r= -0.34, p= .02; r= -0.47, p= .01; and r=-0.33, p= .04 respectively).

The increase of naive CD4+ T-cell count relative to the median of the age-specific reference values was subsequently analysed. (18, 19) Interestingly, when CD4+ T-cell restoration was studied as percentage of normal values, the inverse correlation between

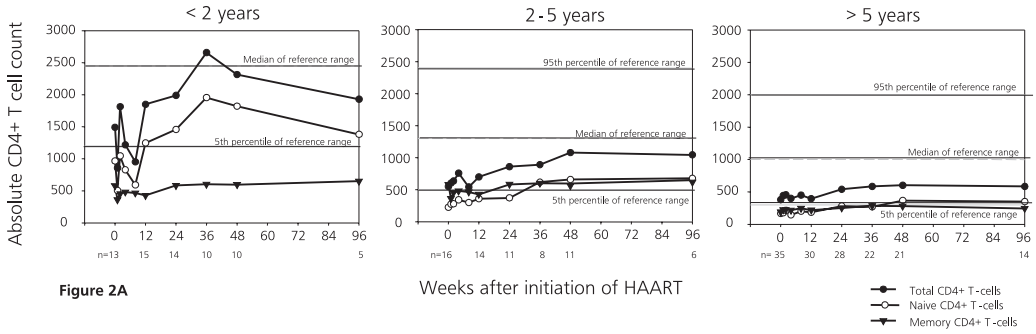


Figure 2A

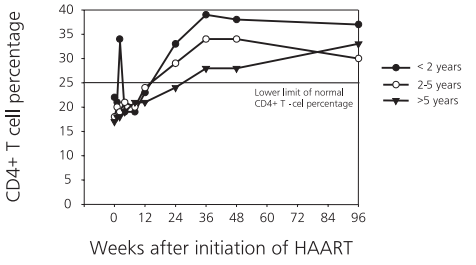


Figure 2B

FIGURE 2A Absolute total, naive and memory CD4+ T-cell responses in three age groups (<2 years, 2-5 years and >5 years). The number of patients which are analysed is depicted.

FIGURE 2B The increase of CD4+ T-cell counts as percentages of total T-cell counts in three age groups (<2 years, 2-5 years and >5 years).

the increase of naive CD4+ T-cell count and age was not observed after 4, 24 (Figure 3B) and 36 weeks.

Therefore, younger children produce more cells in absolute numbers. However, they need to produce more CD4+ T-cells in order to normalise their CD4+ T-cell counts. One may therefore conclude that older children are able to normalise their CD4+ T-cell counts as well as younger children.

CD4+ T-cell response and virologic response

The immunologic reconstitution (absolute CD4+ T-cell counts, CD4+ T-cell counts as percentage of normal, CD4+ T-cell counts as percentage of total T-cell count) was not different at any timepoint in virologic responders and virologic non responders. Figure 4A shows the change from baseline of CD4+ T-cell counts as percentage of normal in responders versus non-responders.

CD4+ T-cell response and baseline CD4+ T-cell count

Strongly immunosuppressed adults have poor immunological recovery.(17) Therefore, we analysed in our patients the relation between baseline CD4+ T-cell counts and the increase of CD4+ T-cells. We observed a inverse correlation between baseline CD4+ T-cell counts and the change from baseline of CD4+ T-cell counts as percentage of normal after 2, 4, 36, 48 and 96 weeks (r= -0.25, p= .05; r = -0.25, p= .05; r = -0.49, p = .001; r = -0.42, p =

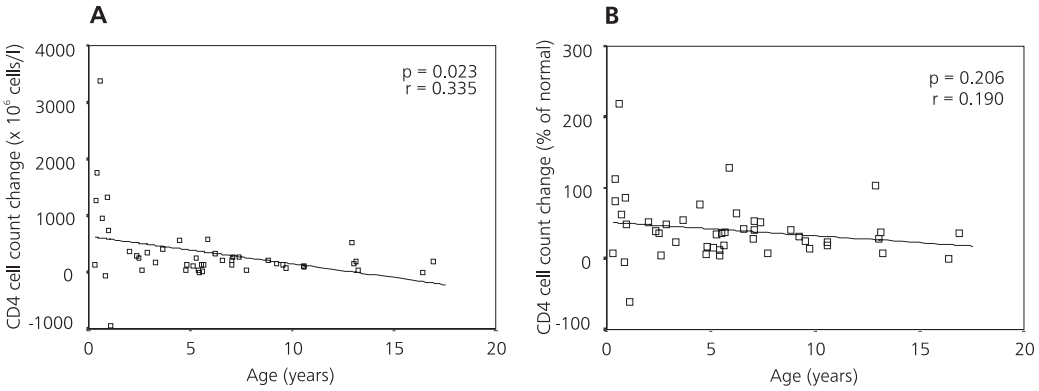


FIGURE 3A Relation between the change of absolute naive CD4+ T-cell count and the age of the children after 24 weeks of HAART. The correlation coefficient was calculated by the Spearman rank-correlation method.

FIGURE 3B Relation between the change of naive CD4+ T-cell counts as percentage of normal and the age of the children after 24 weeks of HAART. The correlation coefficient was calculated by the Spearman rank-correlation method.

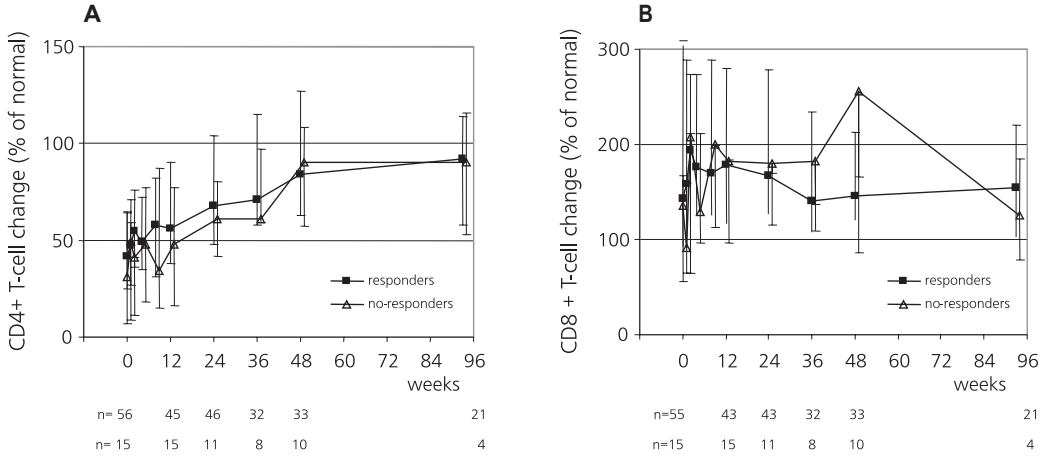


FIGURE 4A Change from baseline of CD4+ T-cell count as percentage of normal in virologic responders versus non-responders.

FIGURE 4B Change from baseline of CD8+ T-cell count as percentage of normal in virologic responders versus non-responders.

.01; and r = -0.41, p = .04 respectively). Thus, children with lower baseline CD4+ T-cell counts showed a larger increase of CD4+ T-cells after the initiation of HAART. Recovery to normal values was seen even in children with very low CD4+ T-cell counts (<10 x 106 cells/L) at baseline.

CD8+ T-cell responses

The median absolute CD8+ T-cell counts, the CD8+ T-cell percentage of total T-cells and the CD8+ T-cell percentage of normal did not change after the initiation of therapy. No significant difference was found between virologic responders and non-responders in CD8+ T-cell counts as percentage of normal (Figure 4B).

T-cell responses to CD3 plus CD28 mAb stimulation *in vitro*

To study functional recovery of T cells during HAART, T cell proliferation *in vitro* was analysed An increase of T-cell responses to CD3 plus CD28 mAb stimulation *in vitro* from a median (IQR) of 1368 (675-2716 (cpm/10^3 T-cells)) at baseline to a median (IQR) of 2325 (1480-3542 (cpm/10^3 T-cells)) after 48 weeks was observed (p=0.06). Expressed as percentages of the median of 3450 healthy adult donors the T-cell response increased from a median (IQR) of 68% (33-134) at baseline to 115% (73-175) at week 48 (p=0.06).

Discussion

In this study the long-term immunologic response to HAART, consisting of one protease inhibitor and two nucleoside analogues, was evaluated in HIV-1-infected children. CD4+ T-cell numbers in HIV-1-infected children on HAART recover more rapidly than CD4+ T-cells in HIV-1-infected adults as has been published by others. (6, 11, 13-16) This good immunological response to HAART has been attributed to the functioning thymus present in young children. Our study is the first in which recovery of CD4+ T-cell counts is related to reference values for lymphocyte subpopulations. In general, absolute CD4+ T-cell counts are used in pediatric studies regarding T-cell repopulation during HAART, since CD4+ T-cells as percentages of total T-cell counts are influenced by the major changes in the number of CD8+ T-cells which are encountered in HIV-1-infected patients (6-8). However, analyses of T cell repopulation in groups of children with different ages are hampered by the fact that CD4+ T-cell counts are highly dependent on the age of the patients. (18, 19) Reference values of younger children (< 2 years) are much higher and have a large range compared to older children (> 2 years). (18, 19) Hence, younger children need to produce larger numbers of CD4+ T-cells to achieve their normal age-related CD4+ T-cell counts. The calculation of CD4+ T-cell counts as percentage of normal absolute values thus results in an independent parameter for the degree of CD4+ T-cell restoration.

Using this method it appears from the data that CD4+ T-cell counts in older children are restored to the same degree relative to their normal values as in younger children. This is in agreement with the correlation between thymic size and the increase in naive CD4+ T-cell numbers (9, 26) , because younger children, who have a larger thymus, need to produce more naive CD4+ T-cells to recover and maintain normal CD4+ T-cell counts. Analyses of absolute CD4+ T-cell counts showed that our data are consistent with the previously observed finding that the repopulation of absolute CD4+ T-cell counts is more rapid and more complete in children than in adults. (6, 11, 13-16) Even children with extremely low CD4+ T-cell counts at baseline did reach normal values during the follow-up period.

Reconstitution of the immune system in these children is predominantly caused by the production of naive CD4+ T-cells. The initial increase of memory CD4+ T-cell numbers as observed in adults, is not seen in children. (7, 8)

In addition to the quantitative improvement of the immune system, T-cell function after stimulation with CD3 mAb plus CD28 mAb also improved. Since proliferation of T-cells was expressed as thymidine incorporation per 10^3 T-cells, circulating T-cells had an increased capacity to proliferate after the initiation of HAART. This indicates that there is also functional improvement of T cells during HAART.

A remarkable finding was the absence of differences between virologic responders and virologic non-responders in respect to immunologic reconstitution despite the long term follow-up of 96 weeks. Similar observations have previously also been reported by others. (13, 15, 22, 27) The phenomenon could be explained by the selection of certain viral variants with resistance to protease inhibitors that have in-vitro impaired replicative capacity. (28) Douek et al. also reported an increase of peripheral CD4+ T-cell counts in both virologic responding and non-responding children on antiretroviral therapy. However, they observed that the recovery of thymic function was affected by the degree to which virus suppression was achieved when thymic function was measured by quantifying T-cell receptor rearrangement excision circles in peripheral blood. (29)

Our results indicate that normalisation of CD4+ T-cell count in HIV-1-infected children on HAART is age-independent, suggesting that thymic function allows the children in all age groups to meet their widely different CD4+ T-cell production demands. Remarkably, HAART had a beneficial effect on immune reconstitution regardless of virological success, even when children were in an advanced stage of HIV-1 infection.

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5 Persistent humoral immune defect in HAART-treated HIV-1-infected children: Loss of specific antibodies against attenuated vaccine strains and natural viral infection

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Abstract

Objective In the pre-highly active antiretroviral therapy era, a loss of specific antibodies was seen**.** Our objective with this study was to describe the loss of specific antibodies during treatment with highly active antiretroviral therapy.

Methods In a prospective, single-center, cohort study on 59 children with HIV-1 infection, we investigated the long-term effect of highly active antiretroviral therapy on the titers and course of specific antibodies against measles, mumps and rubella vaccine strains compared with wild-type varicella zoster virus, cytomegalovirus, and Epstein-Barr virus.

Results During highly active antiretroviral therapy, age-adjusted $CD4^+$ T cells and B cells increased, whereas total immunoglobulin levels declined. Although these children were preimmunized before the start of highly active antiretroviral therapy, only 24 (43%) had antibodies against all 3 measles, mumps and rubella. Antibodies against measles, mumps and rubella were lost in 14 (40%), 11 (38%), and 5 (11%) children who were seropositive at baseline. We also observed loss of varicella zoster virus immunoglobulin G in 7 (21%) of 34, cytomegalovirus immunoglobulin in 3 (7%) of 45, but none of 53 Epstein-Barr virus-seropositive children. During highly active antiretroviral therapy, primary vaccination in 3 patients and 15 revaccinations in those with negative serology demonstrated incomplete seroconversion.

Conclusions Humoral reactivity in children with HIV-1 infection remains abnormal during highly active antiretroviral therapy. Despite immune reconstitution, antibodies against live-attenuated vaccine and wild-type natural virus strains disappear over time in up to 40% of children with HIV-1 infection.

Introduction

HIV-1 infection causes a progressive immunodeficiency as a result of the loss of $CD4^+$ T cells. Consequently, several abnormalities in the B-cell compartment occur. These include a progressive decline in total $CD19^+$ B cells, with polyclonal hyperimmunoglobulinemia,[1,2] impaired reactivity to immunization,[3] and loss of specific antibodies.[4] After successful treatment with highly active antiretroviral therapy (HAART), $CD4^+$ T-cell count increases and a reduction of the hyperimmunoglobulinemia is seen.[5,6] During the first 12 weeks of HAART an increase in absolute B-cell count is found in most patient.[7]

The function of the B-cell compartment is to produce neutralizing antibodies and to maintain serologic memory after primary infection. After measles, mumps, and rubella (MMR) vaccination, lifelong immunity is maintained in healthy individuals. Before the era of HAART, it was found that in children with HIV-1 infection, the initial response to vaccination is weaker and transient compared with healthy children.[3,4] However, the long-term effect of HAART on the B-cell count and function in children is unclear.

Vaccination has lead to a decline in the incidence of measles, mumps and rubella cases in otherwise healthy children, although outbreaks still occur. MMR coverage as well as seroprevalence in the Netherlands is high at 94% with an increase after routine booster immunization at 9 years of age.[8,9]

In this study, we investigated whether the B-cell memory was restored during treatment with HAART. As a surrogate marker for B-cell memory we determined whether the loss of specific antibodies against the components of the MMR vaccination would be influenced by the treatment with HAART. We compared the MMR serology with the humoral response against natural viral pathogens–varicella zoster virus (VZV), cytomegalovirus (CMV) and Epstein-Barr virus (EBV)–and tested whether any loss of specific antibodies would continue despite treatment with HAART and consecutive immune reconstitution and, if so whether this is only seen against live-attenuated viruses or against wild-type viruses as well.

Methods

The Pediatric Amsterdam Cohort on HIV-1 consists of children and young adolescents who are younger than 18 years. Since 1997, patients have received HAART that consists of 2 nucleoside-analog reverse-transcriptase inhibitors and at least 1 protease inhibitor or a non-nucleoside-analog reverse-transcriptase inhibitor. For the present study, we selected all children who had started therapy between 1997 and 2005. The medical ethical committee approved the study for serotyping and (re)vaccination. Caregivers gave written informed consent.

Blood samples

During the routine blood tests, antibody levels against MMR were checked annually, and children were (re)immunized when indicated. The Dutch national vaccination program (Rijksvaccinatieprogramma [RVP]) includes MMR vaccination at the ages of 14 months and 9 years.[9]

Lymphocytes, T-cell subsets, and T-cell proliferation

Numbers of B cells ($CD19^+$), T cells ($CD3^+$), and subsets ($CD3^+CD4^+$, $CD3^+CD8^+$) were determined by standard FACScan procedures, as described before in detail.[8] Age correction for $CD4^+$ and $CD8^+$ T cells and $CD19^+$ B cells was done by dividing the counts by the mean of an age-matched healthy control group.[8]

Plasma HIV-1 RNA determination

Plasma HIV-1 RNA concentration was determined using either Nuclisens HIV-1 RNA QT (Biomérieux, Boxtel, the Netherlands) or Versant HIV-1 RNA 3.0 (Bayer, Tarrytown, NY). All tests were performed according to the instructions of the manufacturers. Because of a different lower limit of detection in the 2 assays, all plasma vial loads (pVLs) < 400 copies per mL were considered as undetectable.

MMR, VZV, CMV and EBV serology

Specific antibodies to measles and mumps were determined by enzyme immunoassay (Virotech, Rüsselsheim, Germany). Serology of measles and mumps is expressed as arbitrary units (AU) per milliliter. An antibody amount of 9.0 AU/mL or more was regarded as positive. Specific antibodies to rubella were determined by Axsym (Abbott, Abbott Park, IL), expressed as IU per mL. An antibody amount of 10.0 IU/mL was regarded as positive. Specific antibodies to VZV were determined by Vidas tests (Biomerieux, Lyon, France). The test values of this assay were converted to IU per milliliter using the conversion factor as determined by van der Zwet et al.[10] An antibody amount of ≥0.139 IU/mL was regarded as positive. CMV antibodies were defined by Axsym assays, expressed as AU per milliliter. An antibody amount of ≥15 AU/mL was regarded as positive. Specific immunoglobulin G (IgG) against the viral capsid antigen (VCA) and against nuclear antigen of EBV was determined qualitatively using respectively the anti-EBV VCA IgG enzyme-linked immunosorbent assay and the anti-EBV nuclear antigen of EBV IgG enzyme-linked immunosorbent assay (Biotest, Dreieich, Germany). All tests were performed following the instructions of the manufacturers.

Seropositivity was defined by the presence of a positive specific IgG after the age of 18 months to exclude any confounding contribution of maternal antibodies in the very young. Serological tests within 3 months after the administration of blood products were excluded from the analyses.

Statistical analyses

Statistical analyses were performed using SPSS 11.5 for Windows (SPSS Inc,, Chicago, IL). All P values were 2-tailed. $P < .05$ was considered statistically significant. Continuous data were analyzed using a Mann-Whitney U test. Categorical data were compared with a

Fisher's exact test. Correlation was tested using the Spearman's correlation test. The mean age-adjusted $CD4^+$ T cells, $CD19^+$ B cells and total IgG were modeled using a mixed model that incorporated repeated measurements. This model handles missing data adequately by estimating the outcome using a first-order autoregressive structure. Differences in these estimates between various levels of the variable were tested for significance using t statistics.

Results

Since 1997, 59 children started treatment with HAART at a median age of 4.3 years; 49% of the children were male, and 24 presented with a Centers for Disease Control and Prevention C classification. Median follow-up since the start of HAART at the time of analysis was 205 weeks (Table 1).

Baseline Serology

Before the start of HAART, only 24 (43%) children had positive antibody titers against all 3 components of the MMR vaccine. Whereas officially reported to be immunized, either according to the national vaccination program or on entry in the health care system, 8 (13%) of the included children who started antiretroviral medication had no detectable antibodies against any of the MMR components and 24 (41%) children had a discordant response against 1 or 2 of the components in the vaccine (Table 1). Of the various components, 35 (63%) children had specific antibodies against measles, 29 (52%) against mumps and 45 (80%) against rubella (Table 2).

Virologic and immunologic response to antiretroviral therapy

On treatment with HAART, the HIV-1 replication was suppressed in most of the treated patients and immunologic recovery occurred in all. After 48, 96, 144 and 192 weeks, 40 (71%) of 56, 35 (66%) of 53, 26 (58%) of 45, and 18 (51%) of 35 children, respectively, had an undetectable pVL of HIV-1 in on-treatment analyses. During 192 weeks of

TABLE 1 Clinical characteristics of the patients at start HAART

Characteristic	N=59
Age (years, median (IQR))	4.3 (1.4-8.8)
Male sex (n (%))	29 (49%)
CDC-classification (n)[a]	
Non-C	35
C	24
Total follow-up on HAART (median weeks (IQR))	205 (124-359)
Number of positive MMR components in previously vaccinated children (Total = 56) (n (%))	
3 positive	24 (43%)
1 or 2 positive	24 (43%)
0 positive	8 (14%)

IQR: Inter quartile range [a] Clinical categories as defined by the US Centers for Disease Control and Prevention [29].

TABLE 2 Immunity at baseline against life-attenuated measles, mumps, rubella and the natural VZV, CMV and EBV infection and loss of specific antibody titers during the treatment with HAART.

	Baseline		Lost	Median time to loss in weeks
	Positive, N	Negative, N	N (%)	
measles	35	21	14 (40%)	103
mumps	29	27	11 (38%)	119
rubella	45	11	5 (11%)	84
VZV	34	25	7 (21%)	161
CMV	45	14	3 (7%)	78
EBV (VCA)	53	6	0	n.a.

treatment with HAART total-IgG declined compared with baseline ($P < .001$; Fig 1A). The decline was most pronounced in the first 48 weeks, stabilizing thereafter. Total IgM also declined during 192 weeks on HAART ($P < .001$). Total IgA showed a non-significant decline ($P = .067$). However, age-adjusted $CD4^+$ T-cell and $CD19^+$ B-cell numbers increased during 192 weeks on HAART (both $P < .001$; Fig 1B), although the increase in B cells was more gradual. When defined as a normalization of the in vitro lymphoproliferative T-cell response on stimulation by the combination of CD3 and CD28 monoclonal antibodies,[11] functional immune reconstitution was complete within 4 to 6 weeks after start of HAART, irrespective of the pVL at start of treatment or during follow-up or age-adjusted $CD4^+$ T-cell count (data not shown).

Serology during treatment with HAART

Of the 35 children with specific antibodies against measles before to the start of HAART, 14 (40%) lost their specific antibodies over time; mumps antibodies were lost by 11 (38%) of 29 and rubella antibodies by 5 (11%) of 45 seropositive children (Table 2). The decline in total IgG and the decline in specific antibodies against MMR were not correlated (r = 0.9, $P = .7$; r = 0.3, $P = .13$; r = 0.2, $P = .24$, respectively). The decline in total IgG and the decline in specific antibodies therefore seemed unrelated to each other.

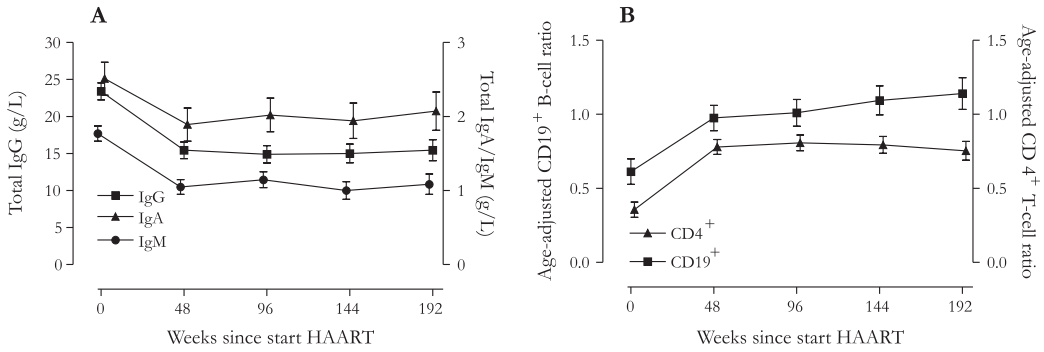

FIGURE 1 Immunological markers during 4 years treatment with HAART. In A, total-IgG, on the left Y-axis, total-IgM and total-IgA on the right Y-axis. In B, age-adjusted $CD4^+$ T cell ratio on the left Y-axis and age-adjusted $CD19^+$ B cell ratio on the right Y-axis. Shown are estimated mean and standard errors of the mean.

After numeric and functional immune reconstitution as a consequence of HAART, the loss of specific antibodies was not anticipated. Several variables were tested for a correlation with the defective humoral B-cell memory response; age-adjusted $CD19^+$ B cell and $CD4^+$ T-helper cell counts, HIV load at start of HAART, age at start of HAART, mode of transmission, and gender. None of these variables correlated with the loss of specific antibodies against all 3 MMR components taken together.

In contrast to the analysis for the MMR components combined, children who lost their measles antibodies were younger than children with sustained antibodies (median: 2.5 vs 6.2 years (P = .04), when these components were analyzed separately. With the

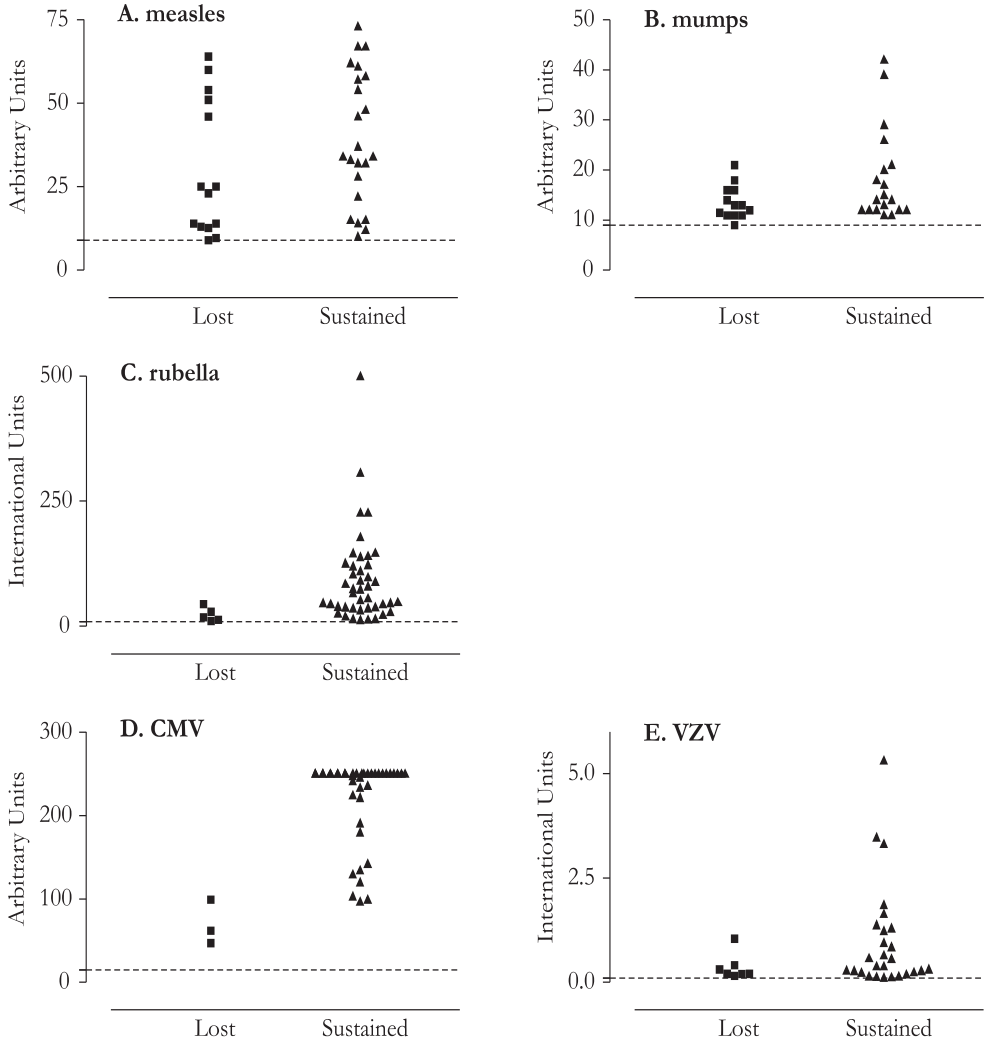

FIGURE 2 Baseline serology in children who lose specific antibodies and children with sustained antibodies. In A. Measles antibodies at baseline (Arbitrary Units). In B. Mumps antibodies (Arbitrary Units). In C. Rubella antibodies (International Units). Rubella titers at baseline were lower in children who lost these specific antibodies than in children who had sustained antibodies (p= 0.005). In D. CMV antibodies (Arbitrary Units). CMV titers at baseline were lower in children who lost these specific antibodies than in children who had sustained antibodies (p<0.001). In E. VZV antibodies (International Units) at baseline.

surprising exception of measles antibodies, almost all children who had HIV-1 infection and showed a loss of specific antibodies during follow-up against mumps and rubella already demonstrated lower antibody titers at baseline. Only in that case of rubella did this difference in antibody titers at baseline reach significance, comparing the children , who had HIV-1-infection and lost these specific antibodies with those who sustained their specific antibody titer (median: 18.1 vs 73.9 IU/mL; (P = .006; Fig 2). These data suggest a weaker response on primary vaccination before the start of HAART.
Looking at vertical (n = 52) and sexual (n = 7) infection separately, 1 of the sexually infected adolescents was found to lose antibodies against a single component of the MMR vaccine during follow-up, whereas 26 vertically infected children lost antibodies against at least 1 component (P = 0.22).

Responses to vaccination during treatment with HAART

During this prospective study, 3 additional children with HIV-1-infection were vaccinated for the first time at the age of 14 months as part of their routine vaccination (RVP). HAART had been started before MMR vaccination and at vaccination immunity was already reconstituted in these 3 children. All responded well and showed complete seroconversion against all 3 MMR components. However, 1 child lost specific antibodies against mumps and another lost both mumps and measles antibodies during continuous treatment with HAART within the subsequent 177 to 244 weeks after vaccination, respectively.
In contrast, 15 children received a second MMR immunization during the treatment with HAART because of the lack of specific antibodies against 1 or more components after their primary vaccination. Revaccination took place before the planned standard vaccination according to the RVP at 9 years of age. Characteristics of these children at the time of vaccination are shown in Table 3. Median age of the children was 7.3 years;

TABLE 3 Characteristics of children revaccinated against MMR during HAART.

N	15	
Age (median (IQR))	7.3	(4.2-9.0)
$CD4^+$ T cell count (median cells/µL(IQR))	1050	(830-1190)
Weeks after start HAART (median (IQR))	119	(47-203)
Weeks between vaccination and serology testing (median (IQR))	48	(19-93)

IQR: Inter quartile range

median $CD4^+$ T-cell count before vaccination was 1050 cells perµL (interquartile range: 830-1190). For these, the median time between the start with treatment and the date of vaccination was 119 weeks. Of the 10 children who were negative for the measles component before HAART, 6 (60%) seroconverted, 8 (89%) of 9 seroconverted for mumps and 4 (80%) of 5 seroconverted for rubella (Fig 3). Antibodies against mumps and rubella , although detectable before revaccination, were no longer detectable after vaccination in 1 child (Table 4).

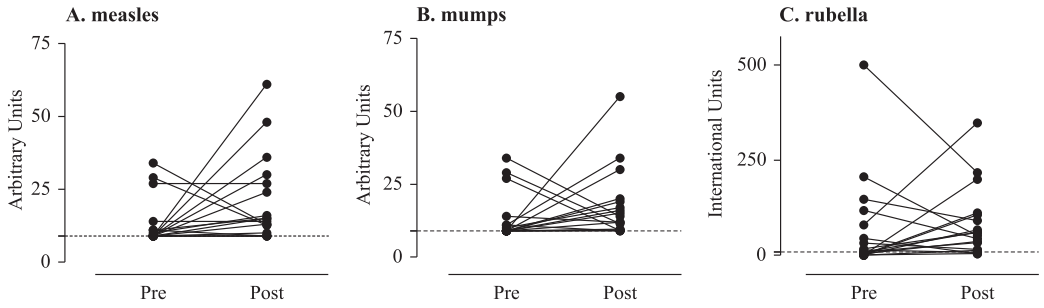

FIGURE 3 Serologic reaction upon booster MMR vaccination. Measles, mumps and rubella serology pre- and post-booster vaccination during treatment with HAART.

Longitudinal analyses of serology against CMV, EBV and VZV

We compared the loss of specific antibodies against the live-attenuated vaccine strains with the humoral response against natural viral pathogens (VZV, CMV and EBV). Specific IgG antibodies against VZV were detected at the start of HAART in 34 children (Table 2). During the treatment with HAART, 7 (21%) children no longer showed detectable levels of VZV antibodies on follow-up. CMV antibodies were detected in 45 children at baseline but were no longer detectable in 3 (7%) children. In contrast, none of the 53 children with EBV VCA antibodies at baseline lost these antibodies during treatment with HAART. CMV antibody titers were significantly lower at baseline in children who had HIV-infection and lost these specific antibodies compared with the children who sufficiently sustained the specific antibody level (median: 62.2 vs. 250 AU/mL; $P < .001$; (Fig 2), but did not differ in age. In contrast, children who lost their VZV antibodies were relatively younger than children with sustained antibodies (median: 4.1 vs. 5.3 years; $P = .04$).

TABLE 4 Effect of re-immunization against MMR during HAART.

	Serology at start HAART, after first vaccination	Serology after re-immunization during HAART
measles	Positive (n=5)	Positive (n=5) Negative (n=0)
	Negative (n=10)	Positive (n=6) Negative (n=4)
mumps	Positive (n=6)	Positive (n=5) Negative (n=1)
	Negative (n=9)	Positive (n=8) Negative (n=1)
rubella	Positive (n=10)	Positive (n=9) Negative (n=1)
	Negative (n=5)	Positive (n=4) Negative (n=1)

Discussion

In the present study, we evaluated the serologic responses against the live-attenuated viral strains of the MMR vaccine as well as naturally occurring common viral infections in children with HIV-1 infection (VZV, CMV, and EBV). We demonstrate that only 43% of the HIV-infected children had specific antibodies detectable against all 3 MMR components at baseline. During treatment with HAART, ~ 40% of the children lost specific MMR antibodies that were present at baseline despite immune reconstitution. Although less frequently, specific antibodies against naturally occurring VZV and CMV also were lost in 21% and 7% of the children, respectively.

The discordant MMR responses before HAART treatment are in line with the findings in the pre-HAART era.[3] However, the low rate of seropositivity and the loss of specific antibodies are in clear contrast with our previous cross-sectional study in > 200 healthy children of mixed racial background, in which we found that > 90% of the children had specific antibodies against all 3 MMR components above the age of 3 years, further increasing to 100% after routine reimmunization at 9 years of age.[8]

In a longitudinal study in > 350 healthy children, specific antibodies against measles and rubella, when regularly measured over a period of 12 and 15 years, respectively, remained positive in 99% to 100% of the children.[12,13] Mumps antibodies were positive in 86% of the same after 9 years of follow-up.[14] These longitudinal data in pediatric control subjects support the relevance of the increased loss of specific antibodies in our cohort of HAART-treated children, irrespective of the immune reconstitution and normalized age-adjusted $CD4^+$ T-cell counts on HAART.

Age was not found to correlate with the loss of specific antibodies in our cohort. Therefore, a relation between time since vaccination and start of HAART is not likely. While on HAART, children with HIV-1-infection also showed vaccine failure upon boosting. Revaccination resulted in seroconversion in only 60 to 85% of the children. Also in the pre-HAART era, it was found that children with HIV-1-infection demonstrated both primary vaccine failure and loss of antibodies after an initial response.[4] In a recent study of 18 children who were treated with HAART and did not have evidence of measles antibodies at baseline after previous immunization, the seroconversion rate was 83% after reimmunization.[15] Eleven children had antibody levels tested after 1 year of follow-up, and only 8 (73%) showed sustained antibody levels. We further extend these data, showing that this loss of antibodies is not unique to measles,[15-17] and is observed for wild-type viruses as well. In total, 27 children (46%) lost antibodies against at least 1 of the MMR, VZV, or CMV strains. Seventeen children lost antibodies against 1, 5 against 2, 4 against 3, and 1 against 4 of these viruses.

Loss of specific antibodies determines an increased risk for serious infection. In areas with a high HIV-1 prevalence, children with HIV-1-infection have high rates of mortality attributable to measles. Although the risk for waning specific antibody levels in communities with a high coverage rate of vaccination may be limited as a result of herd immunity, outbreaks still occur in the developed world.[18] Waning immunity also can be

a potential problem for pregnant women who have HIV-1-infection and come in contact with rubella, VZV or CMV. Furthermore, in a study cohort of 1832 women with HIV-1-infection, an increased risk for shingles was found, irrespective of HAART.[19] After primary infection CMV and EBV persist for life and go into a stage of latency in epithelial tissue and immune cells from where they may reactivate unnoticed.[20] Whereas humoral immunity against EBV was not affected, CMV-specific antibodies unexpectedly were lost in 3 children.

Because the decline in total IgG during HAART and the decline in MMR-specific antibodies are not correlated, these phenomena are unrelated and the consequence of different mechanisms. Hyper-IgG before start of HAART could be produced by low-avidity polyclonal B-cell reactivity, whereas specific memory B-cells are exhausted as a result of inappropriate stimulation as well as the loss of antigen-specific $CD4^+$ T- helper cells.[21,22] During HAART, an increase in total peripheral blood B-cell counts was observed without any change in the relative memory B cell (IgD^- $CD27^+$) fraction (data not shown). However, B-cell memory may not recover functionally, as indicated by our specific antibody data and supported by reports on defective B-cell function in vitro.[5,23,24] Because plasma cells originate from antigen-triggered B cells, a shorter life span of plasma cells in HIV-infected children may result in the decline in specific antibody,[25] which is a completely uninvestigated issue. Similar findings on vaccination responses and the loss of specific antibodies have been reported after chemotherapy and bone marrow transplantation.[26,27] The nature of the immune reconstitution is different, of course, in these settings.

Two important issues warrant additional study. First, VZV-specific cell-mediated immunity has been shown to be unresponsive to HAART.[28] Therefore, waning immunity against VZV may be both humoral and cellular in nature. Although beyond the scope of this study, proliferation tests against MMR and herpes viral antigens over time should give more insight in the biology of the loss of specific antibodies. Second, the order of HIV infection and previous vaccination status may be important for the induction of long-lasting B-cell memory. Studies in adults should be performed to find evidence for the impact of "time of infection".

A shortcoming of this study is the lack of a control group of healthy children who were followed prospectively. Such a control group was considered unethical for the longitudinal nature and multiple venipunctures in healthy children required for direct comparisons. Although MMR serology data in previous longitudinal cohorts of healthy children,[12-14] showed that 86% to 100% of the children maintained specific antibodies during a period of 9 to 12 years when measured every 1 to 3 years, this was at the time of vaccine introduction, and the role of boosting by (subclinical) exposure to the wild-type virus disease is difficult to assess. Using the same assays, our own cross-sectional study in healthy children (admitted to the hospital for unrelated minor trauma or elective surgery) indicated good levels of seroprotection.[8] MMR vaccination is part of the standard vaccination program in the Netherlands, covering > 95% of all children since its national

introduction in 1987. Only rarely, local outbreaks that are confined to small regions occur in the Netherlands, which are considered to have impact neither on the general population nor on the serology against all 3 MMR components.

The loss of specific antibodies as observed in > 40% of children with HIV infection does not seem to occur to the same extent in healthy children. It still remains unclear whether the loss of specific antibodies poses a real threat to HAART-treated children.

Regular testing for the loss of specific antibodies in children HIV-infection with seems mandatory. Repeated immunization may support further the antigen-specific $CD4^+$ T-cell help in maintaining memory B- and plasma cell function, irrespective of HAART.

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6 Viral dynamics after starting first-line HAART in HIV-1-infected children

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Abstract

Background — After starting HAART, the plasma HIV-1 RNA (pVL) declines rapidly to undetectable levels in most treated adults and children. The viral dynamics in children are assumed to differ from those in adults. Therefore viral decay and time to reach a pVL of < 400 copies/mL during the first weeks after starting HAART were studied in a cohort of HIV-1-infected children.

Methods — Viral decay expressed as half-life and time to reach a pVL of < 400 copies/mL in 39 HIV-1-infected children starting HAART were calculated and correlated with age, pretreatment with antiretroviral mono- or duo-therapy, and baseline pVL.

Results — Baseline pVL correlated with age ($r = -0.41$, $p=0.01$). Median half-life of the virus was 2.1 days (IQR 1.8-3.0). No correlation was found between the half-life of the virus and the baseline pVL at the start of treatment, antiretroviral pretreatment or age. Eight children did not reach a pVL of < 400 copies/mL with the first allocated medication regimen. These children were significantly younger than those in whom HIV was successfully suppressed ($p=0.009$). The remaining 31 children reached a pVL of < 400 copies/mL in a median of 8.1 weeks after the start of therapy; time to reach a pVL of < 400 copies/mL was only correlated with baseline pVL.

Conclusions — These results suggest that pVL at baseline correlated with age. HAART was able to suppress pVL below the lower limit of detection in children with a viral decay rate of 2.1 days, similar to adults and irrespective of baseline pVL.

Introduction

HIV-1 infection in children progresses more rapidly compared to adults [1]. This is thought to be caused by the high plasma HIV-1 RNA load (pVL) found in children and their immature immune system. The high pVLs in the very young (10^6 copies per ml and more) are assumed to be reminiscent of those determined in primary HIV infection in adults [2]. However, the spontaneous decline in children is slower than in adults with primary HIV-1-infection [3-6]. These high pVLs are assumed to be a cause of a poorer response to HAART in children compared to adults [7,8].

The pVL is a predictor of disease progression in HIV-1-infected children before starting therapy [5,9,10]. The pVL is also one of the most important outcome measures during treatment with HAART in HIV-1-infected patients.

Modeling changes in pVL after initiation of antiretroviral therapy has provided substantial insight in the dynamics of HIV-1 in adults [11,12]. The decline of pVL after the start of HAART can be best described by a two-phase model. An initial fast decline of more than 99% of pVL in the first weeks after start of therapy represents the decline in free viral particles. After the first few weeks, this is followed by a slower decline, representing the elimination of long-lived HIV-infected cells [13].

Very little is known about the dynamics of HIV-1 in children. A small study with 16 children aged < 2 years showed that infants under the age of 3 months have a slower decline in pVL than children aged between 3 months and 2 years at the start of HAART [14]. The impact of age on HIV RNA response during HAART has been supported by the results of a large UK/Irish collaborative study in 265 children [15]. However, viral decay rates were not defined and a proper explanation for the altered HIV dynamics at young age still remains to be given. When these findings are indeed explained by an inherently reduced viral decay rate at young age, the clinical implications may be very important. It could mean that the moment to start HAART in young children should be reconsidered, if not strictly restricted to those in whom combination therapy is life saving. As a consequence of such unfavorable viral dynamics, HAART may result in the early development of antiretroviral drug resistance in a group of children for whom not that many drugs are as yet registered or available in a palatable drug formulation.

To test whether the early viral dynamics after the start of first-line HAART indeed depend on age, we analyzed the first weeks after the start of HAART in a cohort of HIV-1-infected children. Baseline pVL, early viral half-life, and time to reach a pVL of < 400 copies/mL were studied in our prospective studies in HIV-1-infected children starting HAART at our center.

Methods

Study design and subjects

Data were obtained from the pediatric Amsterdam cohort on HIV-infection (PEACH). This is an ongoing prospective cohort of all children and young adolescents under the age of 18 years, infected with HIV-1 who are treated and followed at our institute. For the present study we selected 39 children who started HAART between 1997 and 2003 of whom we could calculate viral dynamics, having at least two pVL measurements in the first 3 weeks after starting HAART. First-line HAART in children consists of a combination of two nucleoside-reverse transcriptase inhibitors (NRTI), with one non-nucleoside-reverse transcriptase inhibitors (NNRTI) or a protease inhibitor (PI) [16] as in adults.

The doses of the individual drugs given to the patients (Table 1) were as follows; the twice-daily regimen commonly used, consisted of: stavudine 1 mg/kg, lamivudine 4 mg/kg, nelfinavir 45 mg/kg, each twice daily. In some cases, indinavir 800 mg/m^2/day, zidovudine 240 mg/m^2/day, or lopinavir/ritonavir 800/200 mg were prescribed in two doses daily. In case of nevirapine we started with 4 mg/kg once daily escalating to 7 mg/kg twice daily after 14 days; in children older than 8 years we started with 4 mg/kg once daily escalating to 4 mg/kg twice daily.

Our once-daily regimen consisted of: abacavir 16 mg/kg, max 1 x 600 mg, lamivudine 8 mg/kg, didanosine 200- 240 mg/m^2, and efavirenz 14 mg/kg (max 600 mg/day), in one dose each day.

Dose adjustments were performed according to the weight of the children and, in case of nelfinavir [17] and of efavirenz (KML Crommentuijn, HJ Scherpbier, ADR Huitema, TW Kuijpers, JH Beijnen; unpublished data), on consecutive plasma levels.

Day curve drug evaluation of PI and efavirenz was done at day 1 of the regimen. Subsequent evaluations of the drug levels were done with random samples at each visit. None of the children needed dose adjustment based on plasma concentrations below the threshold during the first 3 weeks. Adherence support was intensified when problems with compliance were encountered either by drug levels or by interview.

The Medical Ethical Committee approved the study. All caregivers gave written informed consent. For the present analyses only the first HAART regimen of each child was considered.

pVL determination

pVL was determined either using Nuclisens HIV-1 RNA QT (Biomérieux, Boxtel, the Netherlands) or Versant HIV-1 RNA 3.0 (Bayer, Tarrytown, NY, USA). All tests were performed according to the instructions of the manufacturers. Due to a different lower limit of detection in the two assays, all pVL below 400 copies/mL were considered as undetectable.

Mathematical model

All pVL measurements available from the first 3 weeks after start of HAART were included in the model. When a pVL was higher than the previous viral load, all further measurements were excluded in order to include only the patients that showed a viral decrease. When pVL dropped below the lower limit of detection, only the first measurement below the lower limit of detection was imputed as the lower limit of detection.

The kinetics of plasma HIV-1 RNA during the first 3 weeks after the start of therapy were analyzed by a simple one-exponential model [18]: $V_{(t)} = V_{(0)} * e^{-kT}$

Where V = pVL, k = virus decay rate constant, and T is time (days) since start HAART. For data fitting to the model the least-squared method was used [19]. The half-life elimination of pVL was calculated as follows: $T_{1/2} = \text{Ln } 2/k$.

Statistical analyses

Continuous data were analysed using a Mann-Whitney U test. Categorical data were compared with a Fisher's exact test. Analysis of time to reach undetectable pVL was performed using Kaplan-Meier survival estimates and differences between groups were tested using the log-rank test. The independent effects of age and baseline pVL were analyzed in a multivariate Cox proportional hazards model. All p-values were two-tailed. P-values < 0.05 were considered statistically significant. Statistical analyses were performed using SPSS for Windows version 11.5 (SPSS, Chicago, Illinois, USA).

Results

Patient population

Baseline characteristics of the patients are shown in Table 1. The median age was 4.4 years, 19 (49%) were female, 14 (36%) presented with a Centers for Disease Control and Prevention (CDC) category C event [20], and 13 had been pretreated with mono- or duo-NRTI therapy before 1997.

Until 2002, first-line treatment was a nelfinavir-containing regimen. Thereafter a single-day efavirenz-containing regimen was implemented as first choice. In sum, 26 children were treated with nelfinavir, two with indinavir, one with the combination lopinavir/ritonavir, and nine with efavirenz, all combined with at least two NRTI's.

Baseline pVL

Median pVL was 4.9 log copies/mL (interquartile range (IQR), 4.6-6.0 log copies/mL) at the start of HAART. Children pretreated with mono- or duo-NRTI before the start of HAART had a lower baseline pVL than those who were treatment-naive at start (5.4 vs. 4.8 log copies/mL, p=0.07).

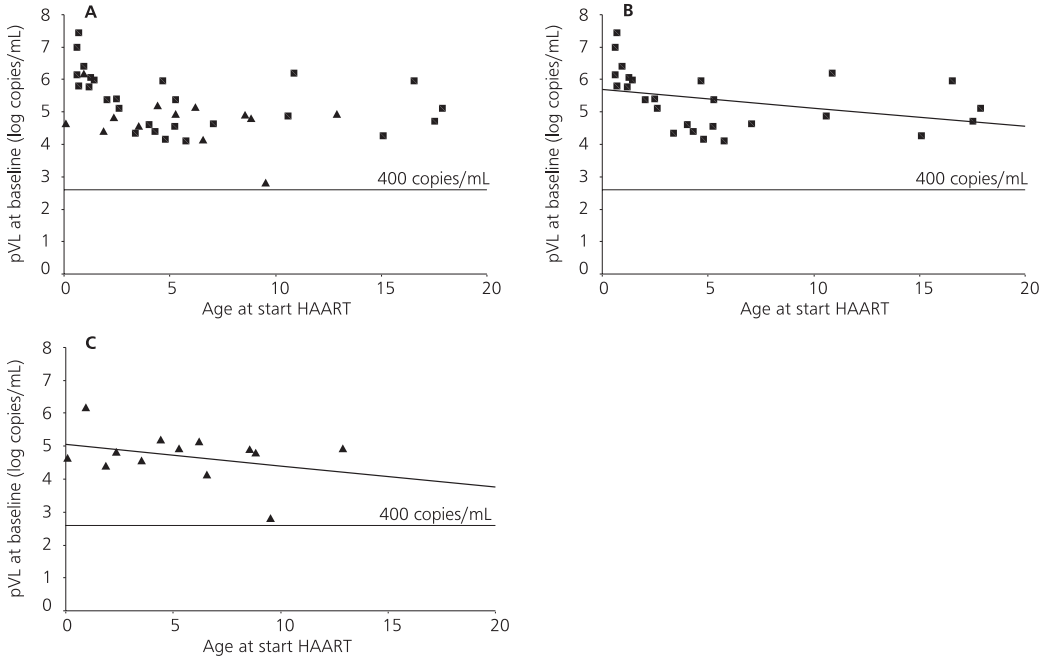
Baseline pVL correlated negatively with age in the total cohort (r= –0.41, n=39; p=0.01) (Figure 1A), or when only the naive patients were taken into account (r= –0.54, n=26; p=0.004) (Figure 1B), indicating that younger children had higher pVL.

TABLE 1 Patient baseline characteristics (n=39)

Median age, years (IQR)	4.4 (1.4-8.5)
Sex (female/male)	19/20
Mode of transmission (vertically/sexually)	37/2
Prior treatment	
None	26
Mono/duo-therapy	13
HAART	
Nelfinavir, 3TC, d4T	25
Nelfinavir, ZDV, 3TC	1
Indinavir, ZDV, 3TC	2
Lopinavir/ritonavir, 3TC, d4T	1
Efavirenz, abacavir, ddI, 3TC	9
Nelfinavir + nevirapine, 3TC, d4T	1
Baseline plasma HIV-1 RNA, median log copies/mL (IQR)	4.9 (4.6-6.0)

IQR, Interquartel range;3TC,lamivudine;D4T,stavudine;ZDV,zidovudine

FIGURE 1 pVL at baseline and age



Baseline pVL negatively correlated with age (r= −0.41; p=0.01) in the total cohort (A), even when only the naive patients were taken into account (B)(r= −0.54; p=0.004). No correlation between age and baseline pVL was found in pre-treated children (C). Squares indicate patients that are treatment-naive; triangles indicate patients that are pretreated with mono- or duo-NRTI therapy. In Figure B. and C. a linear regression line is given.

Viral dynamics

Individual viral decay and half-life of pVL in the first 3 weeks after the start of therapy could be calculated by the least-squared mean method [19]. The time between the first and the last measurement during this period was 2.0 weeks (IQR, 1.1-2.1 weeks). The

median viral decay constant was 0.33 (IQR, 0.23-0.39). Median half-life of the pVL was 2.1 days (IQR, 1.8-3.0). NRTI pretreatment did not influence the half-life of virus during subsequent HAART treatment (pretreated vs. naive; 2.2 vs. 2.1 days; p=0.9). Most importantly, there was no correlation between the half-life of the virus and age at start of HAART (r= –0.05; p=0.8) (Figure 2A) or with the baseline pVL at the start of treatment (r= –0.2; p=0.2) (Figure 2B).

No significant difference in viral half-life between a single-day efavirenz containing regimen (n=9) and nelfinavir containing regimen (n=26) was found in our cohort (2.1 vs. 2.3; p= 0.67).

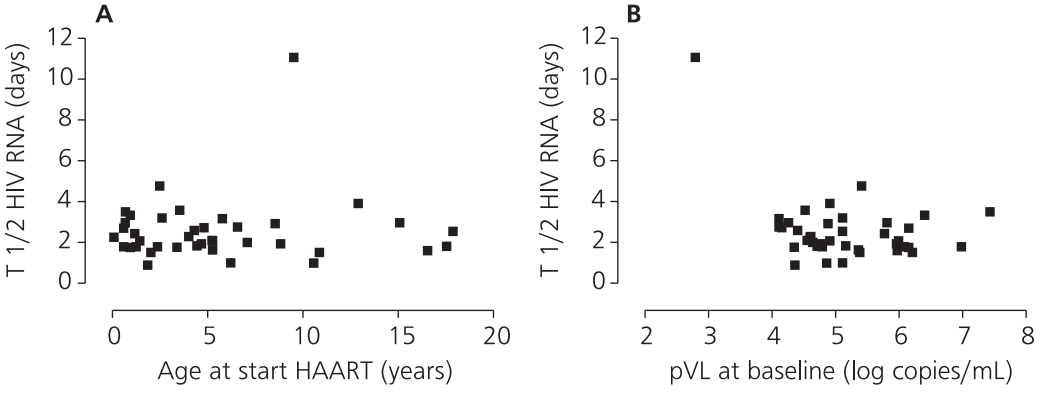
One of the patients had a viral decay constant that was different from the other patients. This 9-year old patient had been pretreated with single NRTI for 173 weeks until the start of HAART. This patient started with a very low pVL (620 copies/mL) and already reached an undetectable viral load at the next visit. Therefore the decay constant is an underestimation of the real situation. However, analyses of the data excluding this patient revealed no substantial differences to our conclusions.

Viral suppression

Of the 39 patients, eight (21%) did not reach a pVL of < 400 copies/mL during the first 48 weeks or had stopped their first prescribed regimen for other reasons. Although clinically and immunologically improved, five children did not reach a pVL of < 400 copies/mL during 48 weeks of continuous use of first-line HAART. The reason for ending medication in the other three children was inconvenience of therapy.

The eight children who failed to reach a pVL of < 400 copies/mL after the start of their first regimen were significantly younger than those who reached a pVL of < 400 copies/mL (median 1.7 vs. 5.3 years, p=0.009). Children who failed to reach a pVL of < 400 copies/mL after the start of their first HAART regimen had a viral half-life during the first 3 weeks comparable with those who did reach a pVL of < 400 copies/mL (median 2.3 days (IQR, 1.8-3.3; n=8) vs. 2.1 days (IQR, 1.8-2.9; n=31); p=0.2). Sex, CDC-

FIGURE 2 Half-life of HIV RNA



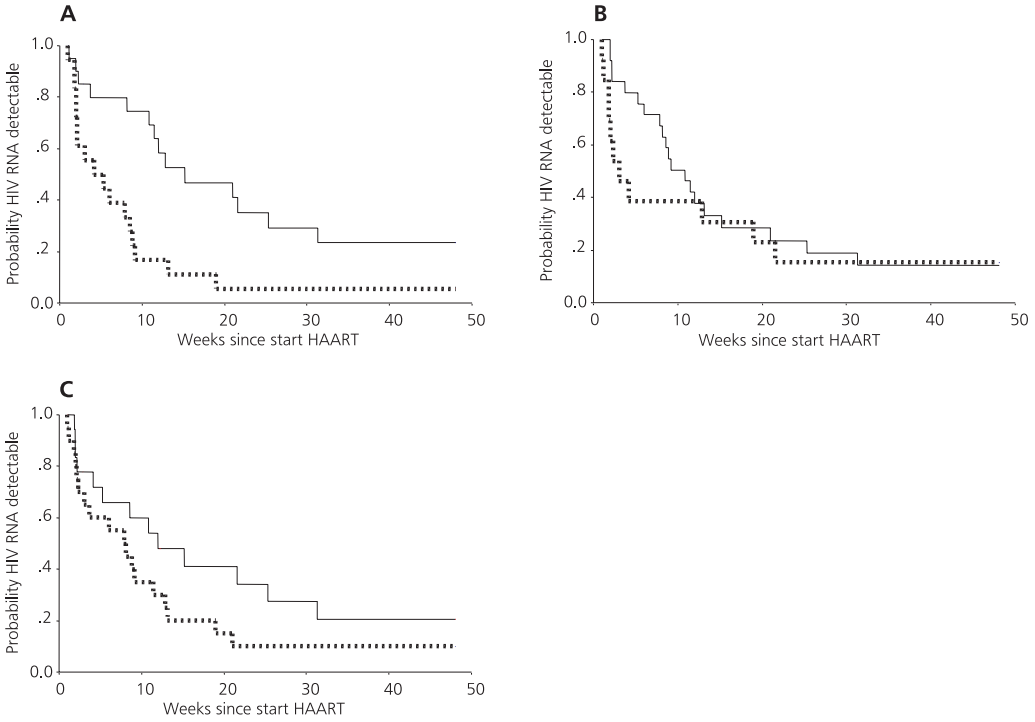
In A, age at the start with HAART and half-life of the virus were not correlated (r= −0.05; p=0.8). In B, Baseline pVL and half-life of the virus were not correlated (r= −0.2; p=0.2). See remarks in Results section on the outlying values.

classification and the number of children that were pretreated with single NRTI before initiation of HAART did not differ between the groups.

The children responding successfully to HAART reached a pVL of < 400 copies/mL in a median of 8.1 weeks after the start of therapy. Kaplan-Meier analyses showed that the time to reach a pVL of < 400 copies/mL was longer in children with a baseline pVL above the median of 4.9 log copies/mL than in children with a pVL below the median (log-rank 8.7; p=0.003) (Figure 3A). Pretreatment (log-rank 0.6; p=0.4) did not result in a prolongation of the time to reach a pVL of < 400 copies/mL (Figure 3B). Age under the median of 4.4 years was associated with a non-significant longer time to reach a pVL of < 400 copies/mL (log-rank 2.1; p=0.15) (Figure 3C).

Because age and baseline pVL are correlated and a non-significant difference is found in time to reach a pVL of < 400 copies/mL in younger vs. older children (median 12.0 vs. 7.8 weeks) we included both variables in a Cox regression analysis. The analysis revealed that baseline pVL (odds ratio (OR), 0.4 [95% CI, 0.2-0.7]; p<0.001) and not age (OR, 1.03 [95% CI, 0.96-1.12] was correlated with the time to reach a pVL of < 400 copies/mL. No interaction was found.

FIGURE 3 Time to virologic response



Kaplan-Meier analyses of the time to reach a pVL of < 400 copies/mL. In A, pVL above (straight line) or under (dotted line) the median (4.9 log10 copies/mL) at baseline. (log-rank, 8.7; p=0.003). In B, children pretreated with NRTI therapy (dotted line) or treatment-naive (straight line) at the start of HAART (log-rank, 0.6; p=0.4). In C, age above (dotted line) or under (straight line) the median (4.4 years) at the start of HAART (log-rank 2.1; p=0.15).

Discussion

We analysed the HIV-1 dynamics in the first weeks after the start of HAART in a cohort of HIV-1-infected children in relation to age, antiretroviral pretreatment and baseline pVL. In this prospective study all children who had at least 2 measurements in the first 3 weeks after the start with HAART were selected in order to reduce the change of selection bias. A median viral half life of 2.1 days was calculated, similar to that established in adults on a three-drug regimen [19]. The viral decay constant and viral half-life were independent of baseline pVL, age at start of HAART or NRTI pretreatment. Hence, the time needed to reach a pVL of < 400 copies/mL in these children was significantly longer in children with a baseline above the median compared to children with a pVL under the median of the group at baseline.

Baseline pVL and the viral decay rate are a reflection of viral turnover. Without treatment, production and degradation of the virus determines the turnover, where the amount of susceptible cells and the fitness of the virus define production and the immunologic host response to the virus the degradation of the virus. When the viral turnover is high, the early decay rate after start of effective therapy will be fast, and, vice versa when low, viral decay will be slow.

Young children tend to have higher pVL than adults. This may be defined by the stage of the infection being acute or subacute in children, whereas most adults present with chronic HIV-infection [4-6]. Although limited to very young children, it is often used to explain the relatively low success rate of treatment of HIV-1 in children, apart from the problems with adherence in this group of patients [7,8] and the need for higher weight adjusted dosage in the very young [21]. None of the children needed dose adjustment based on plasma concentrations below the threshold during the first 3 weeks. The initial intake of medication of these children seemed to be good.

The magnitude of the viral half-life in our cohort was similar to the half-life reported for adults [19,22,23]. Therefore, our data do not support the hypothesis that the decline in pVL is different in children compared to adults. An equal viral decay between sexual infected adolescents and young adults was found in a previously reported study by Wu *et al*. on 115 HIV-1-infected patients [24]. One third of the patients in our cohort was treated with efavirenz in a single-day treatment regimen. No significant difference in viral half-life between the regimens was found in our cohort. In contrast, a more rapid decay of pVL in efavirenz-containing drug regimen when compared to a nelfinavir-containing HAART was seen in the study by Wu *et al*. This difference may relate to the fact that in their study a non-linear mixed-effects biphasic model for the first 6 weeks was used. Instead, we used a linear regression model for the first 3 weeks after the start with HAART, as was previously used by Ho *et al.* [11] and Wei *et al*. [12]. Taking the first 3 weeks together seemed legitimate according to the data presented by van Leth *et al*. for adults [23].

In a previous report, early viral dynamics were described for 12 children under the age of 2 years [14]. It was found that children less than 3 months of age had lower viral decay rates than children between 3 and 24 months of age (0.66 vs 1.03 days, respectively). Children

under 3 months of age are likely to have an increasing pVL as a result of the primary HIV-1 infection rather than the steady state observed later during the chronic stage of infection. This is a possible explanation for the relationship between pVL at baseline and age in our study also. Adult patients with primary infection are known to have lower decay rates than chronically infected patients [25]. In our cohort 11 children were under the age of 2 at the start of HAART of whom only one child started at 2 months of age. We found that all children responded similarly to HAART with respect to pVL. We cannot exclude any difference in viral decay rate below the age of 3 months.

In our cohort a correlation between baseline pVL and time to reach a pVL of < 400 copies/mL was found. In a Cox proportional hazards model we found that this was not confounded by age. In sharp contrast to our findings, age (and not baseline pVL) was recently reported to correlate with the time to reach an undetectable pVL in a large pediatric cohort study [15]. However, the authors used categorical data and the exact relation between age and baseline pVL was not further substantiated. This non-linear dataset of the study may explain that children with a high pVL became undetectable in the same period of time as children with a low pVL.

Instead, we found that the decay rate was comparable irrespective of baseline pVL, suggesting that more time is needed to become undetectable when the pVL is higher at start of HAART. The time needed to suppress pVL below 400 copies/mL was correlated with baseline pVL. If a regimen of antiretrovirals is sufficiently robust, treatment continues to suppress viral replication. To eliminate higher pVL, more time is needed, which would be in line with the finding that age *per se* does not influence the success of HAART when determined after 48 weeks [26]. In line with this is the finding that in adults initial viral decay is not correlated with success after 48 weeks on treatment [23].

On the other hand, the initial decay rate may differ from the HIV suppression in the period after the first weeks. Success of HAART in the longer-term suppression of HIV is dependent on additional factors such as adherence, parental support, drug formulation, drug metabolism, as well as viral mutations that may render the medication less effective. In our cohort, a considerable number of children starting with first-line HAART did not reach undetectable pVL. These children were significantly younger, which may well be related to the aforementioned factors of long-term success. Retrospectively, mutation analysis of the virus before the start with HAART was performed. No mutations associated with the components of the regimen were detected in these children prior to the start of HAART.

Time to reach undetectable pVL was analysed in adults, comparing a three-drug with a five-drug regimen. It was shown that a five-drug regimen suppressed the pVL below the lower limit of detection faster than a three-drug regimen [19]. However, the viral half-life during the first weeks on therapy was comparable between the two treatment groups. Unless adherence or preexistent drug resistance may impact this early stage of treatment, we may believe that the initial viral decay rate during chronic infection would not be different among various HAART regimens.

In conclusion, initial HIV decay after starting HAART was not correlated with age and - expressed as viral half-life of 2.1 days- was similar to the decay rates calculated for adults. Even though baseline pVL correlated with age, the early decay rate did neither correlate with the age of the child nor with the baseline pVL at the start of HAART. Thus, the hypothesis that pVL turnover in children is different from adults cannot be substantiated by our findings.

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7 The pharmacokinetics of nelfinavir in HIV-1-infected children

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Abstract

The authors investigated the steady-state plasma pharmacokinetics of nelfinavir in HIV-1-infected children in a dosage of 30 mg/kg every 8 hours and 45 mg/kg every 12 hours in 12 HIV-1 infected children (aged 2.1 to 10.8 years) participating in an open-label study of nelfinavir in combination with stavudine and lamivudine.

Median values of the primary pharmacokinetic parameters of nelfinavir 30 mg/kg every 8 hours (n=8) and 45 mg/kg every 12 hours (n = 10) were, respectively, for the area under the plasma concentration-time curve over 24 h, 90.5 and 71.9 h·µg/mL, for the trough concentration 1.9 and 1.0 µg/mL, and for the maximal concentration 6.3 and 5.2 µg/mL. Overall, a 7-fold interpatient variability was observed for the exposure to nelfinavir.

Nelfinavir 30 mg/kg every-8-hours or 45 mg/kg every12 hours in HIV-1-infected children generally results in plasma concentrations higher than those obtained in adults. However, due to a large interpatient variability in the exposure, individual dosage adjustments based on plasma concentrations may be necessary for both dosing regimens to ensure optimal treatment.

Introduction

The introduction of protease inhibitors in clinical practice has caused an impressive decrease in morbidity and mortality in HIV-1-infected adults (1,2). Nowadays, the standard of care for the treatment of HIV-1-infection includes one or two protease inhibitor(s) or a non-nucleoside reverse transcriptase inhibitor in combination with two nucleoside analogue reverse transcriptase inhibitors (3). To date, little is known about the use of protease inhibitors in HIV-1-infected children. Results obtained from clinical trials with antiretroviral drugs in adults may not be representative for children because the pharmacokinetics of drugs in children may be different from adults and variable in time, due to the maturation of organ systems involved in drug absorption and disposition (4,5). The protease inhibitor nelfinavir provides a durable antiretroviral response when used in combination with two nucleoside analogue reverse transcriptase inhibitors in HIV-1-infected adults (6). Based on limited data, nelfinavir has also been approved for the treatment of HIV-1-infected children. Nelfinavir is available as a powder and a tablet formulation, and the recommended dosage for patients aged 2 to 13 years is 20 to 30 mg/kg every-8-hours (6). Detailed data on the pharmacokinetics of nelfinavir in HIV-1-infected children are sparse (7-9). Indications for relationships between the pharmacokinetics and pharmacodynamics of protease inhibitors warrant the evaluation of the pharmacokinetics of nelfinavir in children (10,11). Since suboptimal exposure to nelfinavir has been correlated with virological failure, the optimization of plasma concentrations of nelfinavir may be of crucial importance for long-term efficacy (10).

We investigated the pharmacokinetics of nelfinavir in an every-8-hours and an every-12-hours dosing regimen as part of a triple therapy with stavudine and lamivudine in HIV-1-infected children participating in an ongoing, open-label, observational study in the Netherlands. Especially in the pediatric population, every-8-hours dosing is inconvenient because the third dose of nelfinavir has to be administered late in the evening and with a meal or a light snack, often with help from the parents or guardians. Since non-adherence to antiretroviral therapy has been correlated with virological treatment failure, a more practical every-12-hours dosing regimen may be important for sustained viral suppression (12,13).

Materials and methods

Patients

Patients were recruited from the Emma Children's Hospital, Amsterdam, the Netherlands, between October 1997 and February 1999, and participated in an ongoing, open-label, observational study to evaluate the safety and efficacy of combination antiretroviral therapy with nelfinavir, stavudine, and lamivudine in protease inhibitor-naive HIV-1-infected children. The protocol was approved by the institutional review board, and

parents or guardians gave written informed consent. Nelfinavir was administered as tablets (250 mg) or as the pediatric formulation (50 mg nelfinavir per gram of powder, mixed with food prior to administration) in a dosage of 30 mg/kg every-8-hours or 45 mg/kg every 12 hours (maximum, 2,500 mg/d). Stavudine was administered as an oral solution (1 mg/mL) or as capsules (15, 20, 30, or 40 mg) in a dosage of 1 mg/kg every 12 hours (maximum, 80 mg/d) and lamivudine was administered as a liquid formulation (10 mg/mL) or as tablets (150 mg) in a dosage of 4 mg/kg every 12 hours (maximum, 300 mg/d). The first dose of both stavudine and lamivudine was administered simultaneously with the first dose of nelfinavir. Concurrent medication not known to interfere with the metabolism of nelfinavir was allowed on the pharmacokinetic sampling days.

Pharmacokinetic sampling

After a minimum of 7 days on treatment with nelfinavir the children were admitted to the hospital for the assessment of a pharmacokinetic curve of nelfinavir in plasma during one dosing interval (i.e., 8 or 12 h for the every 8 h or every 12 h regimen, respectively). After an overnight fast, patients ingested nelfinavir during a breakfast. For the 30 mg/kg every-8-hours regimen, a total of 12 heparinized blood samples (2.5 mL each) were drawn just before and 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, and 8 hours after the administration of nelfinavir using an IV catheter. At each sampling time, the first mL of blood was discarded to prevent dilution of the samples by heparin. For the 45 mg/kg every-12-hours regimen, two additional samples were drawn at 10 and 12 hours post-ingestion. The blood samples were kept at ambient temperature. After isolation of the plasma by centrifugation (900*g* for 10 minutes at ambient temperature) on the same day, the samples were stored at –30 °C until analysis.

Bioanalysis of nelfinavir

Nelfinavir concentrations in plasma were quantified using a sensitive and validated isocratic, reversed-phase, ion-pair, high-performance liquid chromatographic (HPLC) assay at ambient temperature with ultraviolet detection at 210 nm as previously described (14). Briefly, sample pretreatment consists of a solid-phase extraction procedure using C_2 columns (recovery 70.1 % ± 2.7%). The analytical column is a Zorbax® SB-C_{18} column (75 x 4.6 mm I.D; particle size 3.5 µm) and the mobile phase is composed of acetonitrile plus distilled water containing 25 mmol/L sodium acetate and 25 mmol/L hexane-1-sulfonic acid and adjusted to pH 6.0 (40.5:59.5 v/v). Between-day and within-day precision of the analysis of nelfinavir in human plasma range from 4.0 to 6.4%; the mean accuracy is 98.6%. The lower limit of quantification of the assay is 50 ng/mL. The assay is linear up to concentrations of at least 25 µg/mL.

Pharmacokinetic analysis

Plasma concentration (C) versus time (T) data were analyzed by noncompartmental methods. The highest observed plasma concentration was defined as C_{max}, with the corresponding sampling time as T_{max}. The terminal, log-linear period (log C versus T) was defined by the last data points ($n \geq 3$) by visual inspection. The absolute value of the slope ($\beta/2.303$) was calculated by least squares linear regression analysis. The elimination half-

life ($t_{1/2}$) was calculated by the equation $t_{1/2} = \ln 2/\beta$. The plasma concentration observed at the end of the dosing interval (i.e., 8 or 12 h post ingestion for the every-8-hours and every-12-hours regimen, respectively) was defined as C_{min}. If no sample was obtained at the end of the dosing interval, C_{min} was estimated using the equation $C_{min} = C_{last} \cdot e^{-\beta*(Tend - Tlast)}$, where C_{last} is the nelfinavir concentration in the last sample, T_{end} is 12 or 8 hours for the the every-12-hours and every-8-hours regimen, respectively, and T_{last} is the time interval between ingestion of nelfinavir and the drawing of the last sample. The area under the plasma concentration - time curve ($AUC_{[0-8h]}$ and $AUC_{[0-12h]}$ for the every-8-hours and every-12-hours regimen, respectively) was calculated using the trapezoidal rule from 0 to 8 or 12 hours for the every-8-hours and every-12-hours regimen, respectively. The $AUC_{[0-24h]}$was obtained by multiplying the $AUC_{[0-8h]}$ or the $AUC_{[0-12h]}$ by 3 or 2, respectively. The apparent oral clearance, corrected for body weight, ((Cl/F)/kg; where F represents the oral bioavailability) was obtained by dividing the ratio of the daily dose and the $AUC_{[0-24h]}$ by the body weight. The apparent volume of distribution, corrected for body weight, ((Vd/F)/kg) was calculated by dividing the ratio of the apparent oral clearance and β by the body weight.

Statistical analysis

Statistical calculations were performed with the Statistical Product and Service Solutions (SPSS) for Windows, version 6.1 (SPSS Inc., Chicago, IL, USA). A *P* value ≤ 0.05 was considered statistically significant for all tests.

Results

Patients

Twelve vertically HIV-1-infected children (aged 2.1 to 10.8 years, 5 males, 7 females), who were treated with nelfinavir, stavudine, and lamivudine, participated in this pharmacokinetic study. Two children used nelfinavir in the 30 mg/kg every-8-hours regimen, four children used the 45 mg/kg every-12-hours regimen, and six children were sampled twice, once for each dosing regimen. Median patient characteristics (and ranges) at the time of pharmacokinetic sampling are listed in Table 1.

The median duration of treatment with nelfinavir prior to pharmacokinetic sampling for the every-8-hours and every-12-hours group was 2 and 16 weeks, respectively. For every 8 hours' dosing of nelfinavir, 5 out of 8 children (63%) and for every-12-hours dosing 1 out of 10 children (10%) used the pediatric powder formulation. Concurrent medication consisted of co-trimoxazole (9) and prednisone (1), which are not known to interfere with the metabolism of nelfinavir (15).

Pharmacokinetics

In total, 8 and 10 pharmacokinetic curves were obtained for the 30 mg/kg every-8-hours (mean 29.1 mg/kg; range, 25.3 – 31.4 mg/kg) and the 45 mg/kg every-12-hours (mean 45.7 mg/kg; range, 40.8 – 49.2 mg/kg) regimen, respectively.

TABLE 1 Patient characteristics at the time of pharmacokinetic sampling.

	Median	Range
Age (years)	5.4	2.1 – 10.8
Weight (kg)	19.1	11.2 – 27.8
Length (cm)	110	87 – 133
Body surface area (m^2)	0.74	0.52 – 1.00
HIV-1-RNA (log10 copies/mL)	2.60	2.60 – 4.77
CD4+ lymphocytes (cells/μL)	620	70 – 1,600

The median values (and ranges) of the pharmacokinetic parameters of nelfinavir in a dosing regimen of 30 mg/kg every-8-hours (n=8) and 45 mg/kg every-12-hours (n=10), are shown in Table 2. Figure 1 shows all individual plasma nelfinavir concentrations as measured for the every-8-hours and every-12-hours regimen, and the median plasma concentration- time curves of nelfinavir in both dosing regimens. There was no apparent reason for the the high nelfinavir concentrations in one subject on 30 mg/kg every 8 hours. For six children pharmacokinetic data were obtained from both the 30 mg/kg every-8-hours and the 45 mg/kg every-12-hours dosing regimen. The formulation of nelfinavir (pediatric formulation or tablets) which was administered was not significantly different for the every-8-hours and every-12-hours regimen ($P = 0.27$, Fisher exact test). No significant differences were observed for the $AUC_{[0\text{-}24h]}$, C_{max}, C_{min}, T_{max}, $t_{1/2}$, (Cl/F)/kg, and (V/F)/kg, between the every-8-hours and every-12-hours dosing regimen in these six patients ($P \geq 0.22$, Wilcoxon matched-pairs singed rank test). However, it is important to note that this study was not designed to detect a difference between the two-dosing regimens. For this subset of patients, the individual values for the $AUC_{[0\text{-}24h]}$ of nelfinavir in both dosing regimens are presented in Figure 2. The median values of the $AUC_{[0\text{-}24h]}$ for the every-8-hours and every-12-hours dosing regimen in these patients were 93.6 and 79.4 h · μg/mL, respectively ($P = 0.35$, Wilcoxon matched-pairs signed rank test).

TABLE 2 Values for nelfinavir after multiple after multiple doses in HIV-1-infected children

	30 mg/kg tid		45 mg/kg bid	
Pharmacokinetic parameter	Median	Range	Median	Range
$AUC_{0\text{-}24h}$ (h*μg/mL)	90.5	48.9 – 265.2	71.9	37.7 – 116.8
C_{min} (μg/mL)	1.9	1.5 – 8.0	1.0	0.1 – 3.3
C_{max} (μg/mL)	6.3	3.3 – 14.2	5.2	3.3 – 8.9
T_{max} (h)	2.8	1.8 – 4.8	3.9	1.8 – 7.8
$t_{1/2}$ (h)	3.1	2.5 – 5.9	2.5	1.5 – 6.2
(Cl/F)/kg (L/h/kg)	1.0	0.3 – 1.7	1.3	0.7 – 2.3
(Vd/F)/kg (L/kg)	3.9	2.8 – 9.6	6.0	2.5 – 11.9

$AUC_{[0\text{-}24h]}$ = area under the plasma concentration versus time curve; C_{min} concentration at 8 or 12 h after ingestion of nelfinavir, for the tid and bid regimen respectively; C_{max}, maximal concentration; T_{max}, time to reach C_{max}; $t_{1/2}$, plasma elimination half-life, (Cl/F)/kg, weight corrected apparent oral clearance; (V/F)/kg, weight corrected apparent volume of distribution.

FIGURE 1 Individual plasma nelfinavir concentrations after chronic administration of 30 mg/kg every 8 hours (●) and 45 mg/kg every 12 hours (○) in HIV-1- infected children, and the median plasma nelfinavir concentration- time curves for the every 8 hours (n=8, solid line) and every 12 hours dosing regimen (n = 10, dotted line).

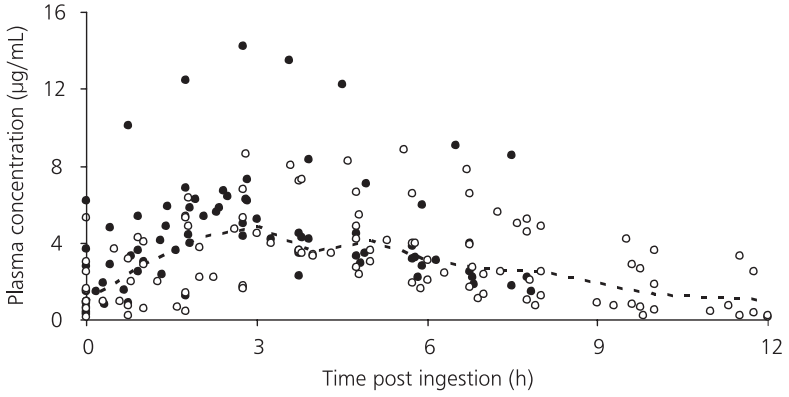
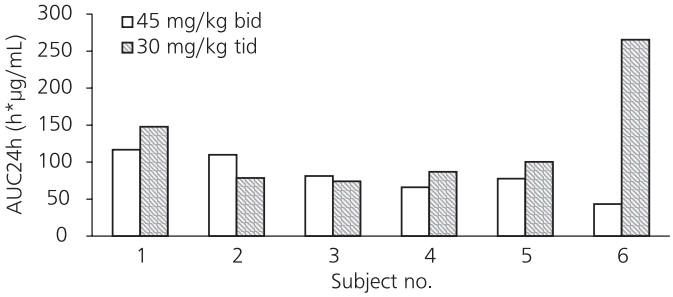


FIGURE 2 Individual values of the steady-state area under the plasma concentration-time curve over a 24 hour period (AUC_{24h}) for nelfinavir in a dosing regimen of 45 mg/kg bid and 30 mg/kg tid in six HIV-1-infected children.



Discussion

This pharmacokinetic study was initiated to investigate the steady-state plasma pharmacokinetics of nelfinavir in a dosage of 30 mg/kg every 8 hours. During the course of the study several children were switched, for practical reasons, to every-12 hours dosing of nelfinavir while maintaining the same daily dose (i.e. 90 mg/kg). Especially in the pediatric population, every-12-hours dosing is more practical and is expected to improve compliance. Knowledge of the pharmacokinetics of nelfinavir in pediatric patients is required to ascertain adequate dosing for sustained antiviral efficacy together with good tolerability. The pharmacokinetics of nelfinavir in a dosing regimen of 30 mg/kg every 8 hours, and in a more practical regimen of 45 mg/kg every 12 hours in children are currently reported.

At the start of this study, nelfinavir and ritonavir were the only protease inhibitors approved for administration to pediatric patients older than 2 years. For the treatment

of HIV-1-infected children unable to swallow tablets, nelfinavir and ritonavir are available as a powder (50 mg/g) or as a liquid formulation (80 mg/mL), respectively. Powder and tablet formulations of nelfinavir have been shown to produce similar plasma concentrations in children (7), which is in agreement with our findings. Nelfinavir was the protease inhibitor of choice for this study in pediatric patients because it is generally well tolerated when administered to adults (6). Recently, amprenavir has been licensed for the treatment of HIV-1-infected children older than 4 years.

It has been shown that the clearance of nelfinavir in children is higher than in adults, when adjusted for body weight (6). Consequently, children are administered a higher dose per kg of body weight, as compared with adults, to achieve similar plasma nelfinavir concentrations (i.e., 20-30 mg/kg for children versus ± 10 mg/kg for adults, assuming an average weight of 75 kg). The apparent oral clearance (Cl/F/kg) of nelfinavir in both dosages in our study is in agreement with previously reported findings in children (i.e., ± 1 L/h/kg) (6,16), and approximately twice the value reported for adults using nelfinavir in the dosage of 750 mg every 8 hours (17). Since the dosage per kg body weight in children is approximately 3 times higher than the dosage for adults, the exposure to nelfinavir in our study is somewhat higher as compared with adults using nelfinavir 750 mg every 8 hours (for comparison, the mean $AUC_{[0\text{-}24h]}$ in adults using this dosage is is 59 h · µg/mL (17)). A marked interindividual variability (7-fold) in total systemic exposure to nelfinavir (expressed by the $AUC_{[0\text{-}24h]}$) is observed in this population. The $AUC_{[0\text{-}24h]}$ ranged from 37.7 h · µg/mL to 265.2 h · µg/mL. The exposure to nelfinavir in one child using the every-8-hours regimen, however, seems to be exceptionally high as compared with the other values, due to unknown reasons. Without the corresponding value for the $AUC_{[0\text{-}24h]}$, there is a 3.9-fold variation in the exposure to nelfinavir. Interindividual variability in the dose per kg of body weight, and the expression and the developmental phase of CYP3A isoenzymes may have contributed to the variation in the exposure to nelfinavir (5).

The median values for the $AUC_{[0\text{-}24h]}$, the C_{max} and the C_{min} for the every-12-hours regimen were 21%, 17%, and 46% lower as compared witht the every-8-hours regimen. The lower C_{min} during every-12-hours administration of nelfinavir is in agreement with observations in adult patients in whom a C_{min} of 1.0 µg/mL and a 0.7 µg/mL has been reported for the every-8-hours and every-12-hours regimen, respectively (6). Our observations are in diasagreement with the data from Schuster et al., (9) who reported a higher exposure to nelfinavir in HIV-infected children for the every-12-hours regimen as compare with the every-8-hours regimen. However, these results may be difficult to compare since the children in the current study were younger and received lower doses of nelfinavir.

Administration of nelfinavir in an every-12-hours dosing regimen might enhance patient compliance, resulting in prolonged efficacy (12,13). In adults 1,250 mg nelfinavir every 12 hours has been proven equally effective and safe as compared with the licensed dosage of 750 mg every 8 hours (18). A crossover analysis in six HIV-1-infected children who used nelfinavir in both the every-8-hours and every-12-hours regimen revealed no

significant differences for any of the pharmacokinetic parameters. It is important to note that these findings are based on observations in only a small number of patients with a marked interpatient variation in pharmacokinetic parameters. However, nelfinavir in a dosing regimen of 45 mg/kg every 12 hours results in trough concentrations below the average trough concentration in adults (i.e., 0.7 µg/mL) using a dosage of 1,250 mg every 12 hours for a considerable number of children (6). In our study, for the every-8-hours and every-12-hours regimen 0 out of 8 (0%), and 5 out of 10 (50%) children reached trough concentrations below 0.7 µg/mL, respectively. To minimize the risk of subtherapeutic plasma concentrations of nelfinavir, it might be necessary to increase the nelfinavir dosage, guided by plasma concentrations, when administered in an every-12-hours dosing regimen (9,16,19).

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8 Population pharmacokinetics and pharmacodynamics of nelfinavir and its active metabolite M8 in HIV-1-infected children

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Abstract

Objectives To develop and validate a population pharmacokinetic model that adequately describes the pharmacokinetics of nelfinavir and its active metabolite M8 in HIV-1-infected children; to define factors involved in the pharmacokinetic variability, which could aid in defining dosing strategies; and to correlate the pharmacokinetics to the treatment response.

Methods Protease inhibitor-naïve, HIV-1-infected children were included. A population pharmacokinetic model of nelfinavir and M8 was developed using NONMEM. Bayesian analysis was used to estimate pharmacokinetic values. A pharmacokinetic-pharmacodynamic analysis was performed to study relationships between these values and the virologic response to therapy.

Results From 38 children 724 nelfinavir and 636 M8 plasma concentrations were available. The pharmacokinetics of both compounds were described simultaneously with a one-compartment model with first order-elimination. Clearance (CL/F) and volume of distribution (V/F) were 32.6 l/h (interindividual variability [IIV]: 31.6%) and 281L/h l (IIV: 29.7%) for nelfinavir and 86.2 L/h (IIV: 43.1%) and 42.3 L/h for M8. No factors could be defined that affected the pharmacokinetics of nelfinavir or M8. The overall virologic response was 78% (HIV-1 RNA <500 copies/ml, on-treatment-analysis). No differences in exposure to nelfinavir and M8 were observed between responders and nonresponders. The only factor distinguishing the two groups was a higher baseline HIV-1 RNA concentration in nonresponders.

Conclusion A model was developed and validated that adequately described the population pharmacokinetics of nelfinavir and M8 in a childhood population. No factors affecting dosing strategies were identified, and no correlation could be demonstrated between the exposure to nelfinavir and M8 and the virologic treatment response.

Introduction

The design of optimal treatment strategies for HIV 1-infected children poses an enormous challenge. The variability in pharmacokinetics is high as a result of the continual maturation of body systems, which complicates the optimal dosing of antiretroviral drugs for children.[1] Moreover, maintaining adequate adherence to highly active antiretroviral therapy can be difficult. Problems such as the intake of evening medication during sleeping time, afternoon medication during school, unwillingness of young children and adolescents to take medication and poor palatability add to the factors that already complicate adherence in adults. In most pediatric studies virologic response rates to highly active antiretroviral therapy are inferior to those in adults.[2] Some studies in which dosages of the administered protease inhibitors (PIs) were adjusted after pharmacokinetic evaluation had superior virologic response rates compared with those in which fixed dosages were used.[3-6]

Nelfinavir was the third PI approved by the U.S. Food and Drug Administration for the treatment of HIV-1-infected children in March 1997. It is available in a pediatric formulation (50 mg/g powder) and in tablets (250 mg). It has shown effective suppression of HIV in combination with two nucleoside reverse transcriptase inhibitor (NRTIs) or with 2 NRTIs and a non-nucleoside reverse transcription inhibitor (NNRTI) and demonstrated good tolerability in children aged 0-17 years. [3-5, 7]

Nelfinavir is metabolized in the liver by at least four different cytochrome P450 (CYP) isoenzymes. The most abundant metabolite of nelfinavir, hydroxyl-*t*-butylamidenelfinavir or M8, is formed through CYP2C19 and has anti-HIV activity comparable to nelfinavir in vitro.[8] Thus, M8 plasma concentrations, in addition to nelfinavir concentrations, are of importance in the antiretroviral activity of the drug. Several studies have examined the pharmacokinetics of nelfinavir in the pediatric population [7, 9-16] Less is known, however, about the pharmacokinetics of M8.[17]

Especially in children, it is important to maintain durable virologic suppression on a certain regimen because treatment options are limited because suitable pediatric formulations are not always available. The goal of this analysis was to determine the population pharmacokinetic values of nelfinavir and M8 and to define factors that influence these values and thereby have impact on the outcome of treatment.

Methods

Patients

Patients were recruited from the Emma Children's Hospital, Amsterdam, The Netherlands. All participated in an open-label observational study to evaluate the efficacy and safety of combination antiretroviral therapy with nelfinavir, stavudine and lamivudine in PI-naive HIV-1-infected children. The protocol was approved by the Institutional Review Board. Pretreatment with NRTIs was allowed. Nelfinavir was administered as tablets (250 mg) or

as the pediatric formulation (50 mg nelfinavir per gram of powder) in a dosage of 30 mg/kg three times daily or 45 mg/kg twice a day. Stavudine was administered as an oral solution (1 mg/ml) or as capsules (15, 20, 30, or 40 mg) in a dosage of 1 mg/kg twice a day and lamivudine was administered as a liquid formulation (10 mg/mL) or as tablets (150 mg) in a dosage of 4 mg/kg twice a day.

Sampling and bioanalysis

After a minimum of 7 days on treatment with nelfinavir a subgroup of the children was admitted to the hospital for the assessment of a pharmacokinetic curve of nelfinavir and M8 in plasma during one dosing interval. During 8 or 12 hours, 12 or 14 heparinized blood samples were drawn, for the three times a day or twice-a-day regimen, respectively. In addition to the full pharmacokinetic curves, blood samples at random time points were drawn at each visit to the clinic for the determination of the plasma concentrations of nelfinavir and M8 in all patients. The time of dosing and sampling was recorded. After isolation of plasma by centrifugation, the samples were stored at –20°C until analysis. The concentrations of nelfinavir and M8 were simultaneously quantified, using high-performance liquid chromatography coupled with tandem mass spectrometry as described previously.[18] The validated concentration ranges were 0.05 to 10 mg/L (= 0.00009 – 0.018 mmol/L) for nelfinavir and 0.01 to 5 mg/L (= 0.00002 – 0.0086 mmol/L) for M8. The measured nelfinavir plasma concentrations were reported to the treating physician.

Pharmacokinetic analysis

The nonlinear mixed effect modelling program (NONMEM) Version V (double precision, level 1.1; GloboMax LLC, Hanover MD) [19] was used to perform the analysis with use of the first-order conditional estimation procedure. The adequacy of the tested models was evaluated using statistical and graphic methods. The minimal value of the objective function (equal to minus twice the log likelihood) was used as goodness-of-fit characteristic to discriminate between hierarchical models using the log likelihood ratio test.[19] A *P* value of 0.05, representing a decrease in the value of the objective function of 3.84 points, was considered statistically significant (χ^2 distribution, degrees of freedom [df] = 1). Standard errors for all parameters were calculated with the COVARIANCE option in NONMEM; individual Bayesian pharmacokinetic parameters were obtained using the POSTHOC option.[19]

Basic pharmacokinetic model

The data for nelfinavir and M8 were described simultaneously with two one-compartment models. The oral clearance (CL/F, in which F represents the oral bioavailability) and volume of distribution (V/F) of nelfinavir and M8 were allometrically scaled for body weight (median weight = 18 kg).[20] With this code, the estimated value of CL/F and V/F represent the values for children weighing 18 kg. The apparent clearance and volume of distribution of M8 were estimated as CL/F/fm and V/F/fm, in which fm represents the fraction metabolized. By including body size a priori into the structural model, other covariates could be explored for their influence on the pharmacokinetics independent of size. [21, 22]

Interindividual (IIV) and interoccasion variability in the different pharmacokinetic values were estimated with an exponential error model as suggested by Karlsson and Sheiner.[23] To minimize the influence of possible nonadherence on the results of this study, the additive component was fixed to a low value in the concentration ranges of nelfinavir and M8. The magnitude of the fixed additive component was determined in relation to the suggested nelfinavir trough concentration [24] and by visual inspection of the individual model predicted concentrations plotted versus the observed concentrations.

Covariate model building

To identify possible relationships between the pharmacokinetics of nelfinavir and its metabolite M8 and patient characteristics, the following covariates were collected: gender (SEX), age (AGE), race (RACE), nelfinavir formulation (pediatric formulation or capsules) (FO), frequency (three times daily or twice a day) (FREQ), nelfinavir dose/kg per gift (AMT/kg) and liver enzymes, including alanine aminotransferase (ALAT, U/L), aspartate aminotransferase (ASAT, U/L), alkaline phosphatase (AP, U/L), and serum total bilirubin (TBR, µmol/L). From a small fraction of patients, some covariates were not available at certain time points, mostly laboratory parameters. To avoid bias, a covariate was included in the model indicating missing data.

A covariate was included in an intermediate model when inclusion of this covariate was statistically significant ($P \leq 0.05$; log likelihood ratio test). All significant covariates were included in an intermediate model. A stepwise backward elimination procedure was carried out to retain only parameters that were statistically significant ($P \leq 0.01$) and relevant. Relevance was reached when the typical value of pharmacokinetic value of interest changed at least 10% within the observed range of that covariate in the population.

Model validation

The bootstrap resampling technique was applied as internal validation of the final model using the software package Wings for NONMEM (by N. Holford, version 222, May 2001, Auckland, New Zealand).[25] Parameter estimates for each of the replicate data sets were obtained. The median parameter values and 95% prediction intervals of the bootstrap replicates were compared with the estimates of the original data set.

Pharmacokinetic-pharmacodynamic and statistical analysis

Children (all PI-naive) with a follow up of at least one year were included in the pharmacokinetic-pharmacodynamic analysis. The analysis was performed on the first year of treatment. Children were divided in two groups: responders and nonresponders. Responders were children with either a detectable viral load at inclusion that became undetectable and stayed undetectable during 1 year of follow up or with an undetectable viral load at inclusion that remained undetectable during a year. Nonresponders were children with a detectable viral load at inclusion that did not decrease to undetectable levels during the first year (or did drop to undetectable levels but became detectable again during the year) or children with an undetectable viral load at inclusion that rose to detectable levels within 1 year. An undetectable viral load was defined as two consecutive occasions with an HIV-1 RNA concentration in plasma below 400 copies/mL. On the

other hand, failure was defined as an HIV-1 RNA concentration in plasma above 400 copies/ml at two consecutive occasions.

First, the baseline characteristics were compared between the responders and nonresponders. A Mann-Whitney test was used for the continuous variables, whereas a χ^2 test was used for categorical variables. Second, it was tested whether differences in exposure to nelfinavir and M8 existed between the responders and the nonresponders (Mann-Whitney test). As a measure of exposure, the median values of the area under the plasma concentration versus time curve during 24 hours (AUC_{24h}) and trough concentrations of nelfinavir, M8 and nelfinavir plus M8 during the study period for each patient were used. The AUC_{24h} was calculated by dividing the dose by the Bayesian estimate of CL/F. Bayesian analysis was also used to estimate plasma trough concentrations (C_{0h}) of nelfinavir and M8 at each sampling occasion.

Statistical calculations were performed with the Statistical Product and Service Solutions (SPSS) for Windows, version 11.0.1 (SPSS Inc., Chicago, IL).

Results

Patients

Data from 41 children were available, consisting of full pharmacokinetic curves and of single plasma concentrations taken at several occasions. Data from three children were excluded from the analysis for noncompliance as reported in the patient's charts. If non-compliance was suspected because of low plasma concentrations (nelfinavir plasma concentration < 0.1 mg/L), both nelfinavir and M8 data at that occasion were disregarded. The final data set consisted of a total of 724 nelfinavir and 636 M8 plasma concentrations from complete pharmacokinetic curves at either three times a day or twice-a -day dosing (27 curves from 19 children) and of plasma concentrations at single time points. Ten samples were excluded because the time after drug intake was unknown. Patient characteristics of the data set are presented in Table I. Eighteen males and 20 females were included in the analysis. Their age at baseline ranged from 0.1 to 17.9 years (median 5.3 years). The median follow-up time was 2.9 years (interquartile range 0.9 – 3.8 years). The mean number of occasions per patient was 13 (range, 1 – 28). Dosages of nelfinavir ranged from 100 to 920 mg three times a day corresponding to 16 to 46 mg/kg three times a day. For the twice-a-day dosing regimen, dosages ranged from 275 to 1750 mg twice-a-day, corresponding to 19 to 78 mg/kg twice-a-day.

Pharmacokinetics

Plasma concentrations of nelfinavir and M8 in the final data set are shown in figures 1A and B. The concentrations varied between 0.1 and 12.08 mg/L (0.002 – 0.02 mmol/L) for nelfinavir, and between 0.05 and 6.26 mg/L (0.00009 – 0.01 mmol/L) for M8, respectively.

TABLE 1 Patient characteristics of 38 human immunodeficiency virus type 1-infected children

Parameter		Median	Range	No. of missing
Age at baseline (years)		5.3	0.1 – 17.9	-
Gender M/F (%)	18/20 (47/53)			-
Weight at baseline (kg)		17.3	3.8 – 65.3	-
Height at baseline (cm)		105	57 – 169	-
Race				
White (%)	7 (18)			
Black (%)	18 (47)			
Latino (%)	10 (26)			
Other (%)	3 (8)			
Clinical chemistry				
Baseline ASAT (U/L)		35	17 – 139	2
Baseline ALAT (U/L)		20	6 – 120	1
Baseline GGT (U/L)		19	3 – 679	3
Baseline AP (U/L)		187	40 – 3350	3
Baseline TBR (µmol/L)		4	0 – 93	5
Creatinine (µmol/L)		27	11 – 54	4
Baseline CD4 cell count (cells/µL)		640	10 – 3340	-
Baseline CD4 cell count (%)		21	2 – 46	-
Baseline CD8 cell count (cells/µL)		1335	90 – 7380	-
Baseline CD8 cell count (%)		46	13 - 74	-
Plasma HIV-1 RNA at baseline (log_{10} copies/mL)		2.94	2.60 – 5.64	-
Number of patients undetectable at baseline (%)	14 (37)			

M = male, F = female, ASAT = aspartate aminotransferase, ALAT = alanine aminotransferase, GGT = gamma-glutamyltransferase, AP = alkaline phosphatase, TBR = total bilirubin

Both nelfinavir and M8 data were best described with a one-compartment model with first-order elimination. The absorption phase of nelfinavir was described by a zero-order process and the duration of the absorption process was estimated (D1).

A model with first-order absorption was tested but proved to be less satisfactory than the model with zero-order absorption. The model was further described with the parameters CL_1/F and V_1/F representing the apparent oral clearance and apparent volume of distribution for nelfinavir for a child weighing 18 kg and with $CL_2/F/fm$ and $V_2/F/fm$ representing the apparent oral clearance and volume of distribution for M8 (for a 18 kg-child). Nelfinavir has a lower apparent clearance and a higher volume of distribution than M8, which results in a smaller elimination rate constant ($k_e = CL/V$). Herewith, the formation of M8 is rate-limiting for its elimination and the metabolite declines with the same half-life as the parent compound.

By including interindividual variability in both CL_1/F and V_1/F of nelfinavir and in CL_2/F of M8 and by including interoccasion variability in the relative bioavailability (F), the model was optimised. The additive error component was fixed to a value of 0.0007 mmol/

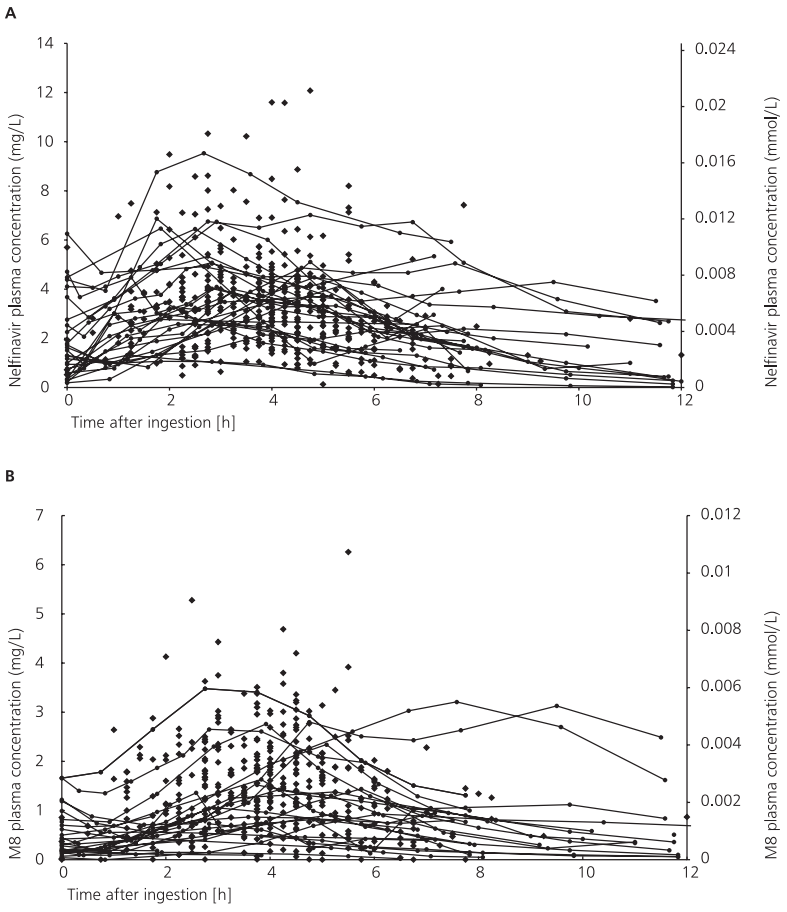

FIGURE 1 Plasma concentration versus time data for nelfinavir (panel A) and M8 (panel B). Solid diamonds (◆) represent plasma concentrations at a single time point, dots connected with hairlines represent full pharmacokinetic curves.

L (= 0.40 mg/L) for nelfinavir and 0.00034 mmol/L (= 0.20 mg/L) for M8, respectively. The magnitude of the fixed additive component is approximately two times lower than the suggested nelfinavir minimum trough level in adults and children.[24, 26] The results of the pharmacokinetic model are summarized in Table 2.

The different covariates of interest were introduced separately into the basic model for D1, CL_1/F, $CL_2/F/fm$ and F. The frequency of nelfinavir dosing (FREQ) and age, categorized at < 2 and > 2 years of age (AGE), both had a significant relation with CL_1/F. The nelfinavir formulation (FO) had a significant relation with D1. The dose per kilogram per gift (AMT/KG) had a significant relation with F. In the multivariate analysis, no factor could be defined that had a significant and relevant effect on the pharmacokinetics of nelfinavir or M8.

Table 2 lists the results of the bootstrap procedure, presented as median and 95% prediction intervals. Median values of the bootstrap analysis were close to the parameter estimate of the original data set and could be estimated with acceptable precision.

Pharmacokinetic-Pharmacodynamic Analysis

From the 38 children in the data set, 28 could be included into the pharmacokinetic-pharmacodynamic analysis. Ten children had a pharmacological or virologic follow-up of

TABLE 2 Final parameter estimates of the pharmacokinetic model

Variable	Final model		Bootstrap analysis
	Est	RSE (%)	Median (95% PI)
D1 (h)	2.92	0.7	2.92 (2.06 – 3.88)
CL_1/F (L/h)*	29.5	5.8	29.6 (26.2 – 33.0)
V_1/F (L)*	296	8.9	289 (237 – 354)
CL_2/F (L/h)*	85.9	8.7	85.9 (72.5-102.0)
V_2/F (L)*	40.2	21.0	39.0 (20.5 – 63.0)
Interindividual variability CL_1/F (%)	28.3	35.6	27.7 (17.0 – 37.1)
Interindividual variability V_1/F (%)	25.8	45.6	24.6 (7.7 – 38.5)
Interindividual variability CL_2/F (%)	45.5	31.7	44.4 (30.3 – 59.4)
Interoccasion variability F (%)	41.6	14.5	41.6 (35.5 – 47.7)
Additive error nelfinavir (mmol/L)	0.0007	-	-
Proportional error nelfinavir (%)	25.8	22.0	25.5 (21.0 – 29.1)
Additive error M8 (mmol/L)	0.00034	-	-
Proportional error M8 (%)	35.5	20.6	35.5 (29.6 – 40.6)
* values apply to a child weighing 18 kg			

D1 = Duration of absorption, CL_1/F = apparent clearance of nelfinavir, V_1/F = apparent volume of distribution of nelfinavir, CL_2/F = apparent clearance of M8, V_2/F = apparent volume of distribution of M8, F = relative bioavailability, Est = Estimation, RSE = relative standard error (as calculated with COVARIANCE option of NONMEM), PI = prediction interval

less than one year. Twenty-two children were classified as responders, which results in a short-term response rate of 78%. Eleven of these children had an undetectable HIV-1 RNA plasma concentration at inclusion, whereas the other 11 children had a detectable HIV-1 RNA plasma concentration (mean 3.1 $\log_{10}$ copies/mL; range, 2.7 – 5.4). Six children did not respond to the therapy. One of them started with an undetectable HIV-1 RNA plasma concentration that became detectable in the first year; the other five had a detectable HIV-1 RNA plasma concentration at inclusion (mean, 3.6 $\log_{10}$ copies/mL; range, 3.2 – 5.6) that stayed detectable. None had been pretreated with PIs other than nelfinavir. No differences in baseline characteristics between the two groups were observed except for the baseline HIV-1 RNA plasma concentration (Table 3). The HIV-1 RNA values were compared only for the children with a detectable level at baseline.

The results of the pharmacokinetic-pharmacodynamic analysis are shown in Table 3. No significant differences could be observed in the exposure to nelfinavir, M8 or in the sum of both. However, a trend was visible toward lower trough concentrations and AUC24h values for M8 in the nonresponders.

TABLE 3 Results of the pharmacokinetic-pharmacodynamic analysis *

Variable	Responders (n = 22)	Non-responders (n = 6)	p-value†
Age at baseline (years)	4.7	4.9	0.96
Gender M/F (%)	13/9 (59/41)	3/3 (50/50)	1.0
Plasma HIV-1 RNA at baseline (log_{10} copies/mL)§	3.1	3.6	0.03
Baseline CD4 cell count (%)	20	21	0.96
Nelfinavir AUC24h (mmol*h/l / mg†h/l)	0.0977 / 55.5	0.1004 / 57.0	0.43
Nelfinavir C0h (mmol/l / mg/l)	0.0027 / 1.53	0.0027 / 1.53	0.72
M8 AUC24h (mmol*h/l / mg†h/l)	0.0392 / 22.9	0.0288 / 16.8	0.07
M8 C0h (mmol/l / mg/l)	0.0012 / 0.70	0.0008 / 0.47	0.06
Nelfinavir + M8 AUC24h (mmol†h/l)	0.1459	0.1352	0.72
Nelfinavir + M8 C0h (mmol/l)	0.0040	0.0037	0.72

* Patients are divided into responders and non-responders. Median values are shown. (responders n=11, non-responders n=5) † Mann-Whitney §§ Only the values > 2.6 log10 copies/mL were used in calculating the median values AUC24h: area under the plasma concentration versus time curve, Coh: trough plasma concentration

Discussion

We developed and validated a model that adequately describes the pharmacokinetics of nelfinavir and its main, equally active metabolite M8 in children. With use of non-linear mixed-effect modeling, we were able to study the pharmacokinetics and the factors with influence on pharmacokinetic values of nelfinavir and M8 simultaneously.

In the end, no factors could be defined with influence on the pharmacokinetics of nelfinavir or M8. In the univariate analysis, several covariates were identified that affected the bioavailability and apparent clearance of nelfinavir. In the multivariate analysis, however, more stringent criteria were applied and none of the factors showed significant and relevant influence on the nelfinavir pharmacokinetics. This might be explained partly by the relationship between the tested covariates and body size, which was included a priori in the structural model. Moreover, diversity in diet might have overruled any effect. Diversity in diet was also identified as a main contributor to the observed pharmacokinetic variability within and between children. The bioavailability of nelfinavir is influenced by the quantity and type of food with which it is administered. Studies have demonstrated that the exposure to nelfinavir is two to three times higher in fed than in fasted subjects.[8, 27] Diet was not controlled in our study, although the advice was given to administer the drugs with food. Adherence also plays an important role in this pharmacokinetic analysis.[28-30] Adherence rates of 57 to 70% have been reported in studies of HIV-infected children[31-33]. We minimized the influence of possible noncompliance on our results by performing a careful data checkout and by fixing the additive component of the residual variability to a value equal to half the desired trough concentration. The final pharmacokinetics values for nelfinavir from our analysis are comparable to the values obtained by van Heeswijk et al.[9] and Hsyu et al.[16]

With use of pharmacokinetic values that were derived from the model, we performed a pharmacokinetic-pharmacodynamic analysis. The virologic response rate was high with 78% of the children responding (on-treatment-analysis) in their first year of treatment. Herewith, the treatment success in this analysis was somewhat higher than in some other pediatric studies using nelfinavir in a fixed dose in combination therapy.[5, 7] The response rate was comparable to the results from studies in which the nelfinavir doses were adjusted proportionally if target values for the AUC, C_{max} or C_{trough} were not achieved.[3,4] Although no target values were applied in this study, the concentrations of nelfinavir and M8 were measured and reported to the treating physicians. This may have positively influenced the response rate.

A higher baseline HIV-1 RNA concentration in plasma was the only baseline characteristic that distinguished the nonresponders from the responders. No correlation could be shown between the exposure to nelfinavir and M8 and treatment outcome. Although a trend could be shown toward a lower M8 trough concentration and AUC_{24h} for the nonresponders, these differences were not visible in the exposure to nelfinavir or nelfinavir plus M8. Because nelfinavir and M8 are both equally active, the meaning of this lower exposure to M8 in non-responders is not clear.

Unfortunately, this study did not yield variables, which could be commonly used to optimize nelfinavir dosing strategies in HIV-infected children. Achieving high rates of adherence clearly is of utmost importance in optimizing treatment outcome. According to the high response rate in this and other studies, pharmacokinetic monitoring could be a good aid. Monitoring M8 concentrations in addition to nelfinavir though does, however not appear to be necessary.

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9 Liver failure in a child receiving highly active antiretroviral therapy and voriconazole

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Abstract

We describe a 10-year-old child with vertically transmitted acquired immunodeficiency syndrome who was receiving antiretroviral combination therapy and died of liver failure after beginning voriconazole therapy.

Introduction

The therapeutic options available to HIV-infected patients have improved since the introduction of HAART. In particular, protease inhibitors have been developed that are substrates of the cytochrome P450 isoenzymes CYP2C9, CYP3A4, and CYP2C19. In vitro studies have indicated that the novel widespectrum triazole antifungal agent voriconazole is also a substrate of these cytochrome P450 isoenzymes [1].We report what is, to our knowledge, the first case of interaction between protease inhibitors and voriconazole.

Case report

We treated a 10-year-old girl with vertically acquired AIDS (Centers for Disease Control and Prevention classification, clinical category 3 [C3]) who was admitted to our clinic with thrush and a severe failure to thrive (weight, 21 kg [2.97 SDs below the mean for the age group]; height, 130 cm [2.12 SDs below the mean for the age group]; weight loss, 3 kg in 4 weeks) [2]. She had been treated from the first year of age with various drug combinations and without any prolonged success. Seven months prior to admission, her therapy was switched to a regimen of amprenavir (22.5 mg/kg b.i.d.), didanosine (120 mg/m2 b.i.d.), nevirapine (4 mg/kg b.i.d.), and a combination of lopinavir (10 mg/kg b.i.d.) and ritonavir (2.5 mg/kg b.i.d.), which she tolerated well. Her HIV RNA level in plasma decreased from 5.46 to 3.11 $\log_{10}$ copies/mL for 5 months, prior to the development of resistance to each class of antiretroviral drugs available. Genotypic resistance was measured by sequencing of the reverse transcriptase gene and the protease gene. Relevant mutations found for the reverse transcriptase gene were: 41L, 44D, 62V, 67N, 74V, 101E, 118I, 181C, 184I, 190A, 210W, 215Y, 219R. Relevant mutations found for the protease gene were: 10F, 20R, 30N, 33F, 36L, 54V, 63P, 84V, 88D, 90M). Her HIV RNA level persisted at ~ 5 $\log_{10}$ copies/mL, with a $CD4^+$ T cell count of 160 cells/mm^3 at the time of admission. The patient's failure to thrive was assumed to result from esophageal candidiasis and inadequate caloric intake as the consequence of painful swallowing. Adenovirus was cultured from the patient's stool specimens; neither diarrhea nor fever was present. She was treated empirically with amphotericin B, administered intravenously for 6 weeks. Concurrent medication consisted of a cotrimoxazole (48 mg/kg b.i.d.) and ranitidine (8 mg/kg b.i.d.). The patient developed moderate renotubular dysfunction and needed supplementation of electrolytes and bicarbonate, whereas clinical symptoms only slightly improved.

Gastroduodenal endoscopy revealed persistent and severe esophageal candidiasis. Cultures of tissue from a biopsy of the esophagus were positive for *Candida albicans,* which had documented resistance to itraconazole and fluconazole but sensitivity to amphotericin B and voriconazole. Intravenous therapy was changed to liposomal

amphotericin B (4 mg/kg) and 5-fluorocytosin (100 mg/kg in 4 doses) for 3 weeks. Because of the lack of any clinical improvement, the antimycotic regimen was changed to oral voriconazole (200 mg b.i.d.), which was used on a compassionate use basis after the informed consent of the patient's parents was given.

One day after the start of voriconazole therapy (day 1), liver enzyme levels had slightly risen (table 1). The patient's liver function deteriorated rapidly within 7 days after the start of voriconazole treatment, and voriconazole therapy was stopped on day 7. The results of serologic testing and PCR blood tests for hepatitis A virus, hepatitis B virus (HBV), and hepatitis C virus (HCV) were negative. A retrospective comparison revealed that, after 2 days, the plasma concentrations of the antiretroviral medication were elevated (lopinavir, 10.4 μg/mL; nevirapine, 7.7 μg/mL; amprenavir, 10.9 μg/mL) compared with the levels during the 6 months prior to admission (lopinavir, 3.9–6.0 mg/mL; nevirapine, 3.5–8.4 μg/mL; amprenavir, 3.5–7.7 μg/mL). The patient had no fever; she was alert and neither showed any neurologic symptoms nor complained about pain. In the presence of progressive liver dysfunction, 5 days after the end of voriconazole therapy, HAART was also stopped; however, irreversible liver failure ensued, followed by hepatic coma. The patient died 28 days after the start of voriconazole therapy. A postmortem investigation was not performed.

TABLE 1 Laboratory results for a 10-year-old child with AIDS receiving HAART who died of liverfailure following treatment with voriconazole.

		Observed value, by day relative to start of VCN therapy								
Laboratory study	Normal range	-50	-42	-12	1	7[a]	9	12[b]	16	28[c]
AST, U/L	0 – 40	53	28	54	127	740	1401	5977	8534	593
ALT, U/L	1 – 45	28	21	23	53	...	209	553	842	334
γ-GT, U/L	0 – 40	100	91	80	101	...	314	693	...	...
LDH, U/L	0 – 220	470	298	390	669	2083	3366	8110	10154	...
Bilirubin, µmol/L	0 – 17	...	5	...	4	...	34	55	101	108
AF, U/L	40 – 120	158	158	104	113	...	215	...	...	...
Amylase, U/L	60 – 220	...	...	...	...	162	...	...	430	...65
CRP, mg/L	<5	9	...	81	117	...	...	72	...	...
Ammonia mmol/L	60 - 220	...	...	...	...	...	...	...	690	...

AST, serum aspartate aminotransferase; ALT, serum alanine aminotransferase; γ GT, γ -glutaramyl transferase; LDH, lactate dehydrogenase; AF, alkaline phosphatase; CRP, C-reactive protein; VCN, voriconazole

[a] Treatment with VCN therapy was discontinued on day 7.

[b] Treatment with HAART was discontinued on day 12.

[c] The patient died on day 28.

Discussion

Although fatal adenoviral infections in HIV-infected children have been described [3], a quantitative PCR assay excluded adenovirus dissemination in the patient's blood. In fact, we believe that our patient died from a noninfectious toxic liver failure caused by drug interaction between one or both of the protease inhibitors the patient was receiving and voriconazole. Extensive antiretroviral therapy can induce some hepatotoxicity in children [4] (although this condition occurs more frequently in adults) in the first months after initiation of therapy and in the presence of concomitant chronic viral hepatitis [5]. However, our patient had never been infected with HBV or HCV and had already used the same regimen of HAART for 7 months without any sign of hepatotoxicity until voriconazole therapy was started. The metabolic impact of the protease inhibitors as a substrate of the cytochrome P450 isoenzymes CYP2C9, CYP3A4, and CYP2C19 has generated concern about hepatotoxicity and the metabolism of other drugs. In vitro studies have shown that voriconazole is a substrate of the aforementioned cytochrome P450 isoenzymes, mostly of CYP2C19 [1]. Protease inhibitors may increase voriconazole plasma concentrations and vice versa. Interactions with reverse transcriptase inhibitors have not been reported [6]. In our patient, the blood levels of the antiretroviral inhibitors were elevated.

In the largest study of voriconazole to date, Walsh et al. [7] concluded that voriconazole was not associated with any increase in the frequency of hepatic abnormalities, compared with liposomal amphotericin B. However, prior use of amphotericin B had not induced any liver dysfunction in our patient. In an efficacy and safety study of voriconazole therapy for invasive aspergillosis, Denning et al. [8] noted abnormalities in the liver function test results of 10–15% of adult patients receiving voriconazole, starting in the first month—and often in the first 10 days—of therapy. In 6 of 22 patients with plasma concentrations >6000 ng/mL, abnormal liver function or liver failure occurred without reported mortality. These effects on the liver are generally reversible after stopping treatment with the drug [7]. In our patient, voriconazole therapy was stopped after 7 days.

Voriconazole exhibits nonlinear pharmacokinetics [9] and may be influenced by certain polymorphisms in the *CYP2C19* gene. Moreover, the concentrations of voriconazole in children are generally much lower than in healthy adults [10]. Measuring the actual plasma concentrations of voriconazole may therefore be of limited value. Thus, even though the concentration of voriconazole was not measured in our patient, a direct and irreversible interaction with HAART most likely occurred.

Our patient's case warrants caution in combining HAART with medication, such as voriconazole, that is metabolised in a similar way. Monitoring drug levels may seem mandatory for lowering the dosage of or stopping medication under certain conditions, but, irrespective of the actual plasma level, will not always prevent acute liver failure from ensuing after medication is stopped, as is illustrated in our case.

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10 HAART in HIV-1-infected children: 10 years of clinical experience - summary and discussion

The introduction of highly active antiretroviral therapy (HAART) has changed the perspectives for adults and children with HIV-1 infection tremendously; resulting in improved quality of life and survival in children and adults with HIV-1 infection (1-6). Before HAART the natural history of HIV/AIDS in children showed a much more rapid progression of disease with a high plasma viral load (pVL), a more profound immune deficiency (depletion of $CD4^+$ T cells) and high risk of opportunistic infections, severe growth retardation and failure to thrive than seen with HAART. Around 23% of HIV-infected infants developed AIDS before the age of 1 year, and nearly 40 % by 4 years of age. Ten percent died in their first year of life and almost 30% before reaching the age of 5 years (7). With HAART available and early diagnosis of infection in infants born to HIV infected mothers, the few infected infants that are being born to infected mothers who had access to HAART or ART during pregnancy now rarely progress to serious disease.
During the last decennium HIV-infection has changed into a chronic disease for both adults and children. However, as well as the positive results of HAART, we are now confronted with the long-term side effects of this therapy. In the pediatric population we have to deal with a growing and developing individual in whom immune defense, the central nervous system, liver function, endocrinologic glands and many other organs still have to mature and adapt to the changing conditions during the various age periods of infancy, childhood and adolescence. Chronic viral infection and its treatment by various antiretroviral regimens have major implications on these developmental issues in children, e.g. growth, bone development and fat metabolism (8,9).
The aim of this thesis was to analyze the long-term efficacy of HAART to suppress HIV-RNA production, to determine the reconstitution of the impaired immune function and responsiveness to vaccination, and to study the pharmacokinetics of the protease inhibitor nelfinavir and the general long-term tolerability of HAART in children with HIV-1 infection.
First, we showed that antiretroviral combination therapy can be as effective in children as in adults. Upon start of HAART, HIV replication can be successfully suppressed and immune reconstitution −defined as an increase of $CD4^+$ T cells− occurred as could be expected from the experience in adult patients. The clinical burden of intercurrent bacterial infections or opportunistic diseases has declined enormously under HAART. On the other hand, side-effects of prolonged use of antiretroviral drugs can not be ignored. Secondly, normalization of $CD4^+$ T cell counts is age-independent, indicating that children of all age-groups can meet their $CD4^+$ T- cell production demand. Nevertheless, humoral reactivity in HIV-1-infected children remains abnormal during HAART. These findings were not correlated to any of the variables tested, such as plasma viral load (pVL) at start

or during treatment failure, age or the disease severity at onset of the start with HAART, and the antiretroviral regimen used.

Despite immune reconstitution, specific antibodies against both live-attenuated vaccine as well as wild-type natural virus strains were either insufficiently generated or disappeared over time in up to 40% of HIV-1-infected children.

Thirdly, individual dosage adjustments based on the plasma concentrations will be required due to a large intra- and inter-patient variability in exposure, uptake, bioavailability and clearance. Dosing errors, unexpected pharmacologic metabolism or drug interactions can occur during HAART, especially in combination with other drugs that may be a substrate for similar metabolic activation or clearance mechanisms. Although repeated monitoring may be necessary for optimization of dosing regimens and successful treatment outcome, one should always be aware of and monitor toxicities.

Viral infection, replication and drugs

During the last decade it has become clear that the cellular and anatomical sites of HIV replication influence the course of the infection, the ability of antiretroviral therapy to reduce viremia, and the establishment of the viral reservoir. This highly mutable virus inserts its genome into the genomes of crucially important cells of the host and, despite therapy, maintains a reservoir of latent HIV within the body (10). The virus has a predilection for activated HIV-specific $CD4^+$ T cells, although other cells are also susceptible to the virus. This tropism for particular cells is determined mainly by cellular receptors to which HIV attaches to enter cells.

The replication cycle can be blocked at several stages. The antiretroviral drugs are directed at specific steps that take place during the HIV-1 replicative cycle. HIV-1 infects susceptible host cell ($CD4^+$ T lymphocytes, macrophages, dendritic cells) by binding of the envelope glycoprotein gp120 to the CD4 surface antigens on major target cell, the $CD4^+$ T cells. After binding to CD4 a conformational change in gp120 allows it to interact more specifically with cellular co-receptors (e.g. CCR5 and CXCR4) in order to enter the cell. This process will result in so-called fusion of the envelope with the plasma membrane of the target cell. RNA is released and undergoes reverse transcription into DNA. This process is catalyzed by an enzyme, called reverse transcriptase. HIV DNA can be integrated into the genome of the cell. This is catalyzed by integrase. Here HIV may persist in a latent state for many years. Activation of the host cells results in the transcription of viral DNA into messenger RNA (mRNA), which is then translated into viral proteins. The new viral RNA forms the genetic material of the next generation of viruses. These proteins migrate to the cell surface and an enzyme, the viral protease, cleaves these proteins into functional HIV-1 particles that will assemble into virions. After virion budding from the cell surface, further maturation of the infectious particle will take place followed by its release.

Two drug classes target reverse transcription, the nucleoside/nucleotide analogue reverse transcriptase inhibitors (NRTI) and the non-nucleoside reverse transcriptase inhibitors (NNRTI). The NRTIs are phosphorylated intracellularly and their triphosphates are

incorporated in the newly synthesized double-stranded viral strains. They lack the 3'hydroxyl group that is necessary for elongation of the viral DNA chain. After their incorporation, the transcription of the viral DNA will be terminated. The NNRTIs bind to reverse transcriptase, downstream of the active site, thereby causing conformational changes which results in the inactivation of the retroviral enzyme.
Protease inhibitors (PIs) bind to the active site of the HIV protease and thereby prevent processing of the so-called structural Gag-Pol polyproteins. In this way, the formation of mature infectious virions can be blocked during the final stages of viral maturation and assembly in the infected cells (11).
New antiretrovirals which interfere in the process of co-receptor binding (CCR5 antagonists) and fusion (enfuvirtide or T-20) and integration (integrase inhibitors) are under investigation in adults and not yet used in children.

Treatment response and virologic failure

The first pediatric HAART regimen used in Amsterdam consisted of a combination of nelfinavir and 2 NRTIs (lamivudine and stavudine), as described in Chapter 2.
From 1997 onward, 39 children were included over the years. Long-term follow-up of these HIV-infected infants and children who were naive to protease inhibitors was determined for about 5 to 7 years. Virologic success was achieved in 74%, 66%, 58%, and 54%, after 48, 96, 144, and 240 weeks following start of HAART, respectively. In this study, children who experienced virologic failure within 48 weeks (or 96 weeks) were significantly younger at baseline compared with the responders (0.8 vs 5.3 years).
The clinical implications of this finding are important in the light of our data and recent discussion on clinical practice and regimen switches, especially where pertinent to the question when to start HAART in young children. Apart from medical issues, all kind of additional psychosocial and economic factors must be considered at the moment treatment will be started.
Recent consensus (in Europe) is to withhold treatment when no serious clinical manifestations or AIDS-defining events have occurred (PENTA Steering Committee; personal communications). The American guidelines for antiretroviral treatment of children with HIV-1 infection recommend antiretroviral therapy, most in particular for infants < 12 months of age, with CDC-A, -B, or -C, and only *consider* ART in asymptomatic infants (N) with CD4% >25% and any pVL. From our data the impression arises that early treatment results in early failure or lack of suppression. Although the precise explanation for this has not been defined, virologic failure in young children is felt to be largely determined by an insufficient drug adherence or under-dosing. Suboptimal suppression in HAART-treated patients has indeed been correlated with non-adherence to the very strict regimens in many different age categories (12-14).
A meta-analysis of virologic outcome data from clinical trials of various HAART regimens found a significant correlation between lower pill-burden and an increased treatment efficacy in adult patients (15).

In a pediatric population compliance can be additionally compromised because of the patient's young age, poor palatability of the medication and dependence on the caregivers. Efforts should be made to increase adherence, especially in the pediatric population, to establish optimal and prolonged HIV suppression and immune reconstitution with the smallest risk possible for long-term side effects.

In order to improve compliance and because of the high prevalence of lipodystrophy in our cohort of HIV-infected children using a 1st-line PI-containing HAART regimen, we developed a new treatment protocol. The protocol was a once-daily regimen containing 1 NNRTI (efavirenz) and three NRTIs (abacavir, lamivudine and didanosine; Chapter 3). Efavirenz was chosen because of its long plasma half-life, allowing once-daily usage. A limited number of studies on once-daily regimens in treatment-naive adults have recently been reported up to 48 weeks, ranging from 50 to 82% virologic failure-free survival (15-22).

The virologic failure-free survival rates in our study were 76% and 67% after 48 weeks and 96 weeks, respectively. No significant difference in efficacy was found between the patient groups taking 1st- and 2nd-line HAART.

As expected, an increasing number of children had developed resistance against antiretroviral drugs applied during the previous PI-containing regimen upon start of the 2nd-line HAART regimen. However, there was no difference in virologic suppression between the HAART pretreated children and those who started naive for antiretroviral therapy.

Although different in study design, the First Pediatric Switch Study showed that children with an undetectable pVL who switched their regimen from PI-containing to PI-sparing HAART, NNRTI can be effectively used to maintain viral suppression (23). Our data now show that children failing their first PI-containing therapy can be treated successfully with an NNRTI-containing 2nd-line regimen.

As was observed in children treated with the PI-containing regimen, all children treated with an efavirenz-containing once-daily regimen again showed a sustained $CD4^+$ T-cell increase, irrespective of virologic suppression.

All children in our study, including the non-responders, showed a sustained immunologic response. Grade 3 and 4 toxicity was observed in only 2 children.

Immune reconstitution

In Chapter 4 we have described the immunologic responses following HAART initiation among 71 children with HIV-1 infection, treated according to 2 prospective, open, uncontrolled national multicentre study-protocols in the Netherlands. Children, naive to PIs, were treated with a PI-containing regimen and followed for 96 weeks. Our data showed a normalization of $CD4^+$ T cell counts in these children independent of their age, further indicating that children could potentially restore their $CD4^+$ T cell counts better and more rapidly than adults, and even in the late stage of infection causing AIDS.

In adults, immune reconstitution following HAART show a biphasic pattern consisting of an initial rapid redistribution of memory T cells and a gradual slow increase in naive

T cells (24,25). Children with HIV-1 infection have a greater capacity to reconstitute the naive $CD4^+$ T cells when compared to HIV-infected adults treated with similar antiretroviral therapy (26). Naive T cell recovery is thymus-dependent. In general, thymic function diminishes with age.

Our observation in children with HIV-1 infection could be attributed to the relatively large thymus and the better thymus function and output in children (27,28). In our study the recovery of $CD4^+$ T-cell counts is related to reference values for lymphocyte subpopulations (29).

Immune reconstitution is predominantly caused by the production of naive $CD4^+$ T cells. In our studies also the T cell function improved as determined by proliferative capacity after stimulation with CD3 plus CD28 monoclonal antibodies. This T cell proliferation is a very rough *in vitro* measure for antigen receptor (CD3)-dependent function, combined with the CD28-mediated co-stimulatory signals necessary for full activation.

Remarkably, both virologic responders and non-responders reacted similarly with respect to immune reconstitution, either when defined as a normalization of $CD4^+$ T cell counts or as *in vitro* T cell proliferation.

Discordant responses to HAART (which means a sustained $CD4^+$ T cell response in combination with virologic failure, or vice-versa, a persistently low $CD4^+$ T cell count with optimal viral suppression) have been reported in ~30% of HIV-infected adults treated with HAART as well as in children (30-32). In adults, virologic and immunologic characteristics remain unclear. Piketty *et al* showed in the immune-responding, but virologic failures (IR+VF) that the proportion of memory $CD4^+$ T cells and the expression of activation markers on T cells were higher and the production of the cytokine IL-2 remained lower as compared to the full-responders (32). There is evidence that viruses recovered from so-called IR+VF patients, may harbor multiple drug-resistance mutations and have decreased fitness in thymic tissue that may permit the regeneration of T cells despite the persistently elevated pVL (33).

Maybe due to the viral resistance to PIs, a selection of viral strains into less-replicating strains with reduced fitness took place in the non-responding children. In theory, this could result in a reduced risk of immunological exhaustion. Further research is needed and planned to explore this possibility in retrospect.

The clinical implications of immune reconstitution are such that primary or secondary prophylaxis against *Pneumocystis jiroveci* (previously known as *carinii*) pneumonia, *Mycobacterium avium*, cytomegalovirus can be discontinued. Whether the condition of immunological failure despite viral suppression represents a risk factor for the long-term incidence of opportunistic diseases −e.g., tuberculosis or malignancies− remains uncertain (34).

Vertically infected children are exposed to HIV during pregnancy, delivery and breastfeeding at a moment that their immune system is still developing. The maturation of the immune system continues after birth. At birth, infants have relatively high numbers of T cells and they are mainly naive in function and phenotype. When children become

older the T cells reach adult values and undergo a memory effector function in *vivo* upon exposure to environmental antigens and infections during childhood (30,35).
An important difference between adults and children is that adults become infected *after* their immune system is already mature and they have already been exposed to so many other infections before the immune system gets eroded.
Although children have the possibility to a rapid immune reconstitution, the impact of early immunizations and (co-)infections has not been studied extensively in children.
It is known that the loss of $CD4^+$ T cells in HIV-infected children coincides with abnormalities in the B-cell compartment, such as a progressive decline in total $CD19^+$ B cells with a polyclonal hyperimmunoglobulinemia (36,37), impaired reactivity to immunization (38) and loss of specific antibodies (39).
After successful treatment with HAART, $CD4^+$ T count increases and a reduction of the hyperimmunoglobulinemia is seen (40,41). During the first 12 weeks of HAART an increase in B cell numbers is found in most patients (42).
The function of the B-cell compartment is to produce neutralizing or high-affinity binding antigen-specific antibodies and to maintain serologic memory after primary infection. Starting in infancy, children receive multiple immunizations (i.e., DKTaP, MMR, Hib, HBV) and contract many childhood infections while growing up, such as chickenpox, CMV or EBV.
In Chapter 5 we have demonstrated that specific antibodies to the viral components of the MMR vaccine were gradually lost in HIV-infected children, even during prolonged treatment with HAART. Although pre-immunized before the start of HAART, only 43% had antibodies against all three MMR components. Antibodies against each component were lost in 40% (measles), 38% (mumps) and 11% (rubella) of the children who were seropositive at baseline. Also regarding common natural wild-type infections, a loss of VZV-specific IgG (21%) and to a lesser extent CMV-specific IgG (7%) was observed, but in none of the 53 EBV-seropositive children.
Infection with VZV can be very dangerous in immunocompromised patients (43). Therefore, it is recommended to immunize HIV-infected children with live-attenuated VZV vaccine if seronegative or without a clinical history of prior chicken-pox (44), except in severe immune-compromised patients, because of the risk of dissemination of the attenuated vaccine strain used (45). The finding in our study that 21% of the pediatric patients lost their VZV-specific antibodies confirms this recommendation. Although HIV-infected children do not generally experience a severe course of wild-type chickenpox, VZV vaccination in healthy children has also reduced the incidence of herpes zoster (HZ) considerably (46). This advantage may be of particular value to HIV-infected patients, who indeed regularly suffer from HZ episodes (47).
During HAART, primary vaccination in 3 patients and revaccination in 15 pre-immune children with negative serology after earlier vaccination demonstrated incomplete seroconversion in 60% for measles, 89% for mumps and 80% for rubellavirus. This has

also been reported by others and may to some extent depend on antigen-specific T cell dysfunction (47,48).

Thus, even during cellular immune reconstitution, antigen-specific immune reactivity remains strongly impaired in HIV-infected children when compared to HIV-negative controls. Immunization success in the healthy population in the Netherlands is large with serologic responses in more than 93-98% of the children (National Institute of Public Health and Environmental Protection [RIVM], Bilthoven, The Netherlands). Apparently, the $CD4^+$ T cells are not able to maintain stable and protective serological levels as has been seen in healthy children with the exception of EBV-specific antibody levels. It is as yet unclear, also not from our data, whether the B cells are affected in their specific responsiveness by HIV-1 itself or indirectly by the dysfunction or depletion of a pool of antigen-specific $CD4^+$ T cells.

It has been reported that the abnormalities in B-cell responsiveness were not only due to impaired $CD4^+$ T cell help, but also intrinsic to perturbations in the B cell compartment itself (49). The impaired responsiveness of B cells on *in vitro* stimulation correlated to the pVL. Reduction in pVL was shown to improve B-cell responses on various stimuli *in vitro* (50). Direct $CD4^+$ and $CD8^+$ T cell responses against a VZV vaccine seem to be comparable to those obtained in healthy children [Bekker, Scherpbier, Kuijpers, submitted]. When confirmed in larger study cohorts and for different antigens, these *in vitro* readouts can explain the lack of severe clinical reactions after administration of such live-attenuated vaccines (e.g. MMR or the VZV-Oka strain) and the decline of opportunistic infections in the era of HAART.

Viral dynamics

In Chapter 6 the viral decay and time to reach a pVL < 400 copies per mL during the first week of HAART was described in HIV-infected children.

HIV-1 infection in children progresses more rapidly compared to adults (51,52). This may be partially explained by the higher pVL and the immature immune system in children compared to adults (53). Often the higher pVL is suggested to explain the reduced virologic effectiveness of HAART at young age. Viral decay after the start of HAART is used as a measure of the viral turnover during HAART. In our study baseline pVL correlated with age; the median half-life of the virus was 2.1 days (IQR, 1.8-3.0) at all ages, strikingly similar as that found in adults.

No correlation was found between the half-life of the virus and the baseline pVL at the start of HAART, antiretroviral pretreatment or age.

On the other hand, 8 of the 39 children did not reach a pVL < 400 copies per mL and again these children were significantly younger than those in whom HIV was successfully suppressed. The remaining 31 children reached a pVL of < 400 copies per mL in a median of 8.1 weeks after start of HAART.

From these data we may conclude that the pVL in young children was not higher as a result of a higher viral turnover. Our hypothesis is that the high pVL in infants can be explained by the immaturity of the immune system in these very young children making

more target cells available for the virus (54), as well as by the still poorly developed immunological capacity to counter the viral infection by an HIV-specific cellular immune response, or −maybe− the quantity of the infecting inoculum.
Finally, Veazey *et al* showed in SIV infection, that the gastrointestinal mucosa (gut-associated lymphoid tissue [GALT]) plays an important role as an initial site for viral replication during primary infection (55). Children may harbor more HIV, because they have relatively more lymphoid tissue per kg body weight compared to their plasma volume. This compartmentalization theory is difficult to prove in children for technical and ethical reasons.

Clinical experience on growth and side-effects

Besides opportunistic infections (e.g., esophageal candidiasis, PCP, CMV infections and reactivations), central nervous system involvement (HIV-encephalopathy), growth retardation and failure to thrive are common features in untreated HIV-infected children (51,56,57).
The etiology of this HIV-related growth retardation is complex (e.g., low caloric intake, the chronic viral infection itself, abnormal function of the thyroid gland, disturbed growth hormone-somatomedin axis, altered lipid metabolism and abnormal resting energy). Growth seems to be one of the most sensitive indicators of disease progression in children with AIDS and the absence of growth indicates a poor prognosis, even in children treated with antiretroviral therapy (58-61). Disease progression is also related to $CD4^+$ T cell count and viral load (52,60). Treatment with HAART has a great impact on these parameters and growth. Already in the pre-HAART era a short-term beneficial effect of NRTIs on height, weight and head circumference was observed (62,63).
In our cohort of HIV-infected children treated with nelfinavir and 2 NRTIs, the pretreated children had not profited in this respect from the previous use of antiretroviral therapy. As is described in Chapter 2, the height-for-age z-score gradually increased to a plateau after the first year of HAART, but never reached the mean of the general mixed Dutch population and the weight-for-height z-scores showed a remarkable increase. The latter increase was mainly in the first 24 weeks after the start of HAART, irrespective of the level of virologic suppression (as was found with the numeric immunologic recovery as well). Thus, treatment with HAART has a major impact on both $CD4^+$ T cell count and growth retardation, reaching a plateau within 2 years of treatment (64).
In Chapter 3 we described the once-daily efavirenz-containing regimen with a backbone of 3 NRTIs. Children naive to antiretroviral therapy had significant lower height-for-age z-scores at baseline than children on 2^{nd}-line HAART. The naive group showed a distinct increase in the first 48 weeks but did not reach the level of the 2^{nd}-line HAART group. Weight-for-age z-scores increased almost to normal levels, although both groups showed distinct patterns. The 2^{nd}-line HAART group showed a higher baseline that remained more stable (due to prior treatment). Weight-for-height z-scores remained stable in the treatment-naïve and the 2^{nd}-line HAART group.

When HAART is stopped for reasons of drug-toxicity or virologic failure, it is still unclear for how long the recovery of growth or immunologic function can persist. This may of course be relevant for further treatment options. For instance, should we continue with HAART in case of virologic failure to sustain the improved clinical conditions of normalized growth and immune parameters? Or should we stop anyhow after the initial recovery has been reached and viral suppression occurred within the first 24 to 48 weeks to spare antiretroviral treatment options before adherence will fail, viral drug-resistance mutations occur or clinical side-effects present?

Side-effects

The virologic success of HAART certainly promises a longer life-span for HIV-infected children, but the metabolic consequences of this therapy are of serious concern. Concomitantly, morbidity from the long-term effects of HAART in adults as well as in children has grown in importance (11,64,65). Among all the complications, lipodystrophy, dyslipidemia, insulin resistance and osteopenia are the most concerning effects of prolonged use of antiretroviral therapy.

In Chapter 2, we described in nearly 30% of the children, treated with a PI and 2 NRTIs, clinically evident lipodystrophy after a median of 49 months. One of these children was extensively pretreated with mono-or dual NRTI therapy.

The effect of a most apparent distortion of their physical appearance in young children and teenagers needs no further comment regarding the risk on failing compliance.

In our once-daily study, evaluating an efavirenz-containing regimen plus 3 NRTIs as backbone, no lipid abnormalities were observed. Children starting naive for HAART had significant lower non-fasting blood levels of total cholesterol than the 2nd-line HAART group at baseline; all children were below the cut-off of 6,5 mmol/L (95th percentile in our hospital). During HAART total cholesterol increased. However, children with 2nd-line HAART remained stable; the naive children showed an increase within the first weeks towards the 2nd-line HAART values. The follow-up period (median, 69 weeks; IQR, 39-122 weeks) in this group is too short to expect neither any effect on fat distribution nor any improvement in those who showed manifestations of clinical lipodystrophy before switching to 2nd-line HAART. Lipodystrophy seems especially common with D4T (in combination with a PI).

In a study by Rhoads *et al* significant elevations of HDL (as well as LDL) was demonstrated in children on HAART (65). The authors concluded that the rise in cardio-protective HDL may represent a positive effect of treatment.

Medical literature contains numerous recent studies in adults related to these complications (66-68). However, the pediatric literature on this subject is still rather sparse (11). Estimates of the prevalence of lipodystrophy range from 2 to 84% in HIV-infected adults (66,68) and from 1 to 43% in HIV-infected children (70-73).

HIV infection, AIDS and antiretroviral therapy has been associated with bone fragility fractures, because of decreased bone mineral density (BMD). In HIV-infected adults osteopenia occurs in 3 to 50%, and osteoporosis in 3 to 21% (66,74-76). The pathogenesis

of the bone abnormalities remains unclear, it is potentially multifactorial, possibly involving HIV, ART, and other non-treatment factors.
Premature bone loss is particularly relevant in vertically infected children because of the lifelong administration of antiretroviral therapy. A physiological peak bone mass will never been achieved in these children. Therefore, children should be monitored carefully for these side-effects by objective (anthromopometric) measurements and objective techniques such as dual energy X-ray absorptiometry (DEXA) to assess the fat distribution and bone density (11,70,73). Regimens should be tested for these side-effects and any impact of these drugs in infants, children and adolescents should be considered carefully. To date, we are collecting data on BMD (by DEXA of the hip and lumbar vertebra, as well as X-rays of the wrist), fat distribution (DEXA) and lipid profiles in blood of our patients. Fractures, often related to trauma, have been reported in adults with HIV-1 infection. Also Perthes disease (avascular necrosis of the femoral head) has been observed in children and adults treated with HAART (11,76).
The 2 HAART regimens described in this thesis did not show many clinically relevant side-effects other than lipodystrophy. One cachectic boy with severe electrolyte disturbances due to intractable diarrhea, died during the study period but −as far as we can tell− under conditions unrelated to the study medication.
Toxicity can, however, also occur rather unexpectedly, as described in Chapter 9. Here we describe a 10-year-old HIV-infected girl, extensively treated with combination antiretroviral therapy, who died of liver failure after beginning voriconazole therapy. Voriconazole exhibits nonlinear pharmacokinetics (77) and may be influenced by certain polymorphisms in the *CYP2C19* gene. Plasma concentrations of voriconazole were not measured in this patient. Other infectious agents, possibly related to toxic liver-failure were excluded and a direct and irreversible interaction of HAART and voriconazole seems most plausible to have occurred in this patient. Co-infection with HBV and HCV are recognized in adults as important risk factors increasing the chance of progression of disease (78). In our PEACH studies we have diagnosed HBV co-infection in 2 children, who show until now stable HIV suppression by once-daily 2nd-line HAART. HCV was not observed in any of these children [unpublished data].

Pharmacology

In Chapters 7 and 8 the pharmacokinetics (PK) of the protease inhibitor nelfinavir and the active metabolite M8 in HIV-1-infected children has been described.
As mentioned before, optimal suppression of HIV is necessary for successful HIV treatment in children and adults. Children are using HAART, composed of the same antiretroviral drugs as adults, although often these antiretroviral drugs have not been registered for their administration to young children. Hence, dose recommendations are often not available.
In children, factors such as poor palatability of medication, the absence of pediatric formulations, complex dosing schedules, physiological maturation of many organs (gastro-intestinal tract, liver enzymes and kidney function in particular) and changes in

distribution of drugs in the body, due to age-specific differences in water content, plasma proteins and permeability of specific compartments for drugs, play an important role in the pharmacokinetics of drugs (79).
Since plasma levels of PIs have been related to virological efficacy, this may have important consequences for the success of treatment (79-83). For a substantial number of antiretroviral drugs and especially in young children dose recommendations are not available.
Based on limited data, nelfinavir has been approved for the treatment of HIV-1 infected children. We investigated PK of nelfinavir in an every-8-hours and an every-12-hours dosing regimen as part of a triple therapy with stavudine and lamivudine. Since non-adherence to ART has been correlated with virologic treatment failure, a more practical every-12-hours dosing regimen may be important for sustained viral suppression (12).
In our study we observed a 7-fold inter-patient variability for the exposure of nelfinavir (expressed by the $AUC_{[0\text{-}24h]}$. When dosed at 30 mg/kg every-8-hours or at 45 mg/kg every-12-hours, nelfinavir generally resulted in plasma concentrations in HIV-infected children that were higher than those obtained in adults.
A review by Fraaij *et al* showed that most of the pharmacokinetic studies performed include small groups of children with a high inter- and intra-patient variability for the pharmacokinetic data of all the available antiretroviral drugs (82). Like others (83-87), we also found a large inter-patient variability in the exposure, and individual dosage adjustments based on plasma concentrations are necessary for both dosing regimens to ensure optimal treatment.
The PK of nelfinavir was extended to its active metabolite M8 in Chapter 8. With the use of the developed model, the pharmacokinetic results were correlated to the treatment response. The PK of nelfinavir and M8 could be adequately described with a one-compartment model with first elimination for both compounds.
The pharmacokinetic-pharmacodynamic analysis between responders and non-responders was neither significantly associated with age, plasma HIV-RNA load, $CD4^+$ T cell count at baseline, gender, nor by nelfinavir or M8 $AUC_{[0\text{-}24h]}$ or their trough concentrations in plasma. The only relevant variable in PK parameters was the frequency of dosing. Moreover, no correlation could be shown between the exposure to nelfinavir and M8 and the virologic treatment response. More in general, these and other data leave us with the question whether there is any proof or reason that urges close monitoring for improving long-term treatment outcome.

Future perspectives

The introduction of HAART changed the perspectives of HIV-infected adults and children tremendously. The combination antiretroviral therapy has improved the quality of life and survival in children and adults. Also in the prevention of mother-to-infant-transmission (MTCT) HAART has decreased the transmission rate to 1% or less in the developed world. The access to HAART in the developing countries is still very low and should be extended to all HIV-infected patients in these countries. Initiatives to ameliorate the

treatment of HIV-infected patient, the prevention of MTCT and in general the care in the third world must be supported by the developed countries. The developing countries should benefit of the improvements made in the developed countries. This implicates also the production of cheaper antiretrovirals and in respect to children more child-friendly formulations. Fortunately, many initiatives have already been taken (such as the implementation of HAART, training of medical staff and counseling of patients), but their continuation should be guaranteed. In the developing world the many tribal or national wars and bad harvests unfortunately intervene regularly.

This thesis has demonstrated that long-term optimal suppression of HIV and immune reconstitution in HIV-infected children is possible and safe with HAART, even in pre-treated children with a positive effect on growth.

European and American guidelines for the antiretroviral treatment of HIV-1-infected children recommend as initial therapy 2 NRTIs with either 1 PI, or 1 NNRTI (88,89). The American guidelines, made by National Pediatric and Family HIV Resource Center (NPHRC), Health Resources and Services Administration (HRSA), and National Institutes of Health advise to initiate therapy in all infants. The World Health Organization (WHO) has made treatment guidelines for resource-limited settings which are more clinically based and if possible, uses $CD4^+$ percentages (90). However, the higher virologic failure rate in young children, the frequent occurrence of lipodystrophy and other side-effects on the long-term during HAART, as well as the recent debate on clinical practice, regimen switches, and the major issue of when to start with HAART in young children, urges doctors and scientists to perform more research and come with the best and most practical answers for further reconsiderations of the present guidelines.

Adherence interventions should go shoulder to shoulder to obtain optimal treatment and prevention of the occurrence of (multi)-resistant viruses. Research in the field of adherence policy and the effect of simplification of therapy regimens needs further exploration, like directly observed therapy (DOT).

In order to prevent non-compliance and therapy failure we have introduced, in collaboration with Institutions for Child Protection and Home Care, a protocol for DOT at home by a home-nursing team. Since we started the protocol, virologic failure has indeed decreased (unpublished data).

Individual dosage adjustments based on plasma concentrations, due to a large intra- and inter-patient variability in the various drug exposures may be required for optimal dosing regimens and successful treatment, although this remains to be shown.

Pharmacogenetic studies may add to understand the lack of clear correlations between short-term virologic suppression and plasma levels of antiretrovirals.

In this respect, further research is strongly needed to look for the causes and development of the lipodystrophy syndrome and its relation to drug levels.

Although HAART gives a general immune reconstitution, the antigen-specific immune reactivity remains impaired in HIV-infected children as described in this thesis. Therefore vaccination policies in HIV-infected children and adults should implement closer

monitoring (protective) levels of specific antibodies to vaccinations and repeated boosting at regular intervals whenever necessary. Special attention should be given to people coming from tropical areas, since these people are may be non- or undervaccinated as such and may be less exposed to VZV as well. In particular pregnant women should be tested for protective levels of MMR and VZV, and revaccinated after delivery to avoid congenital transmission during a following pregnancy and be protected against potential childhood virus infections at adult age, often following a more severe course.
The effects of HAART on child development and their functioning at school is a rather neglected part of HIV research and should be more developed. On the other hand, it may be hard to discriminate between the influence of the medication used versus family conditions such as housing and the financial situation and the many psychosocial stress factors that may impact the psychologic development of HIV-infected children.
Treating HIV-infected children should be done in a structured way so that the research performed will indeed be able to improve the quality of care for HIV-infected children. Multidisciplinary teams, consisting of HIV-specialized nurses, social workers, psychologist, pharmacists, research physicians and pediatricians trained in HIV are obligate tools for good clinical practice. Contact with physicians, treating adults, like infectious diseases specialists and gynecologists are very important in the care of HIV-exposed and -infected families and the transition of HIV-infected adolescents.

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Nederlandse samenvatting

Inleiding

Het humaan immunodeficiëntie virus (HIV) is de verwekker van AIDS (Acquired ImmunoDeficiency Syndrome). HIV heeft al meer dan 40 miljoen mensen geïnfecteerd over de gehele wereld. Het allerzwaarst zijn de niet-geïndustrialiseerde derde-wereldlanden getroffen door deze pandemie.

HIV infecteert $CD4^+$ T lymfocyten. Deze cellen spelen een belangrijke rol in de afweer als een sturende cel in vele afweerreacties die ons tegen binnendringende micro-organismen (voornamelijk virussen, intracellulaire bacteriën zoals de tuberkelbacil, en schimmels) helpt te beschermen. Ze komen voor in de lymfklieren, neus- en keelamandelen, lymfeweefsel in de darmen en de milt. HIV infectie leidt uiteindelijk tot een achteruitgang in het aantal CD4+ T cellen met als gevolg een ondermijning van de afweer. Zozeer dat iemand behalve geïnfecteerd ook zeer ernstig ziek kan worden van vrij banale micro-organismen, de zogeheten opportunistische infecties.

De behandeling van HIV-geïnfecteerde kinderen heeft in de afgelopen 10 jaar een enorme ontwikkeling doorgemaakt door de introductie van 'highly active antiretroviral therapy' (HAART). Tot vóór 1997 werden kinderen met één of twee antiretrovirale middelen behandeld uit de groep van non-nucleoside reverse transcriptase remmers (NRTIs), waarvan AZT het eerste en meest bekende middel is.

In 1996 werd bij volwassenen een nieuwe groep van virale remmers geïntroduceerd: de protease-remmers (PIs). De combinatie van twee (of meer NRTIs met één PI maakte het mogelijk om HIV op verschillende punten in de vermenigvuldigingscyclus van het virus te remmen.

Bij kinderen werd in 1997 de eerste combinatie therapie met PIs gestart. Hierdoor veranderde het 'natuurlijke beloop' van de HIV-infectie bij kinderen enorm. Vóór de komst van deze combinatie therapie ontwikkelde 23% van de HIV-geïnfecteerde kinderen AIDS binnen het eerste levensjaar en ongeveer 40% rond het vierde levensjaar. Tien procent overleed vóór het eerste jaar na de geboorte en 28% vóór het vijfde jaar.

Sinds de introductie van HAART is AIDS een chronische infectieziekte geworden, waarbij kinderen en volwassenen met HIV veel minder ziekteverschijnselen (morbiditeit) ontwikkelen, en het aantal personen dat aan de ziekte overlijdt (mortaliteit), enorm is afgenomen. Bij kinderen is ook het aantal ziekenhuisopnames fors afgenomen.

De behandeling van een HIV-infectie bij kinderen speelt zich nu voornamelijk poliklinisch af. Op de polikliniek worden de kinderen elke 3 maanden gecontroleerd en aan het begin van de antiretrovirale behandeling vaker. De antiretrovirale medicatie wordt op maat gegeven aan de kinderen, dwz de dosering wordt per kilogram lichaamsgewicht of lichaamsoppervlakte berekend en aan de hand van de hoeveelheid in het bloed gemeten medicijn (bloedspiegel) zonodig aangepast.

De behandeling van kinderen vereist een multidisciplinaire aanpak; naast gespecialiseerde HIV-verpleegkundigen, bestaat een behandelteam uit maatschappelijk werk, psychologen, en kinderartsen die gespecialiseerd zijn in infectieziekten en afweerziekten.
De samenwerking tussen de verschillende disciplines is zeer belangrijk. Apothekers zijn belangrijk voor de bepalingen en interpretatie van de data voor eventuele aanpassingen van de doseringen. Virologen helpen om de hoeveelheid virus te volgen in het bloed en de gevoeligheid van het virus voor antiretrovirale therapie te bevestigen en te volgen tijdens de behandeling. Verder versterken speciale onderzoeksartsen het team voor het uitvoeren van wetenschappelijk onderzoek, welke noodzakelijk is voor een optimale behandeling van kinderen met HIV.
De samenwerking met de volwassen disciplines (internisten gespecialiseerd in infectieziekten, gynaecologen, psychiaters, maatschappelijk werkers en HIV-consulenten) is belangrijk, omdat zij de behandeling van kinderen na hun 18^{e} levensjaar gaan overnemen en meestal ook de ouder(s) van de kinderen onder behandeling hebben. Veelal zijn naast het kind één of meerdere gezinsleden geïnfecteerd. Pubers kunnen ook via seksuele contacten geïnfecteerd raken en zijn in dat geval de enige binnen het gezin. Veel gezinnen leven in omstandigheden die extra 'mantel-zorg' vergen en reeds in het verleden 'littekens' hebben opgelopen.
In de vier HIV-behandelcentra voor kinderen in Nederland werkt men min-of-meer volgens ditzelfde behandelingsprincipe.
HAART heeft de overdracht van moeder naar kind (mother-to-child-transmission [MTCT]) aanzienlijk verminderd. In de geïndustrialiseerde landen bedraagt deze nu minder dan 1%, wanneer de HIV-geïnfecteerde moeders tijdens de zwangerschap HAART krijgen. De hoeveelheid virus wordt dan ondetecteerbaar en daarmede de kans op overdracht naar het kind zeer laag. Aansluitend worden de kinderen behandeld gedurende 4 weken met antiretrovirale middelen en wordt de moeders ontraden borstvoeding te geven. Wanneer de HIV-geïnfecteerde moeder vlak voor de bevalling nog een detecteerbare hoeveelheid virus in haar bloed heeft, wordt een keizersnede gepland vóórdat de bevalling zelf op gang komt (electieve sectio caesarea).
In de ontwikkelingslanden ligt het overdrachtspercentage vele malen hoger, in sommige Afrikaanse landen rond de 40%. In deze landen wordt nu op kleine schaal antiretrovirale therapie met goedkopere combinatie-preparaten ingevoerd, meestal alleen rond de bevalling zelf. Voor de preventie van de moeder-kind-overdracht in de derde wereld wordt kortdurend een behandeling met één of twee antiretrovirale middelen gegeven aan moeder en/of pasgeborene. Veel onderzoek wordt verricht om uit zoeken, wat in de derde wereld de beste en de meest voordelige behandelingen zijn. Aangezien veel vrouwen hun kinderen met de borst voeden in de derde wereld, is op dit gebied veel onderzoek gaande. Het is inmiddels gebleken dat in de Afrikaanse setting het beter is alléén borstvoeding of alléén flesvoeding te geven. Wanneer men deze voedingen door elkaar geeft is de kans op overdracht veel groter. Men veronderstelt, dat bij gemengde voeding,

er slijmvliesbeschadiging van de darmwand zou kunnen ontstaan, waardoor het HIV gemakkelijker via de darmwand naar binnen zou kunnen treden.
Na de euforie van HAART worden we nu ook geconfronteerd met de nadelen van de behandeling. Het aantal kinderen, met name jonge kinderen die falen op HAART, dwz bij wie HIV niet goed onderdrukt wordt door de ingestelde therapie, is nog steeds aanzienlijk. Met behulp van de polymerase kettingreactie (PCR) kan men het aantal HIV-RNA strengen in het bloed bepalen als maat voor het aantal virusdeeltjes in het bloed. Wanneer deze boven een bepaalde waarde komen (cut-off of detectiegrens), wordt het virus aantoonbaar. Men noemt dit 'detectable'.
Men streeft ernaar om het virus tijdens HAART 'undetectable' te houden (< 50 of < 400 HIV-RNA kopieën/mL, afhankelijk van de gevoeligheid van de test in combinatie met de hoeveelheid bloed dat men testen kan). Daarnaast beoogt men met HAART het aantal afweercellen, de eerder genoemde $CD4^+$ T cellen te verhogen, zodat de patiënt minder kans heeft op infecties. Zoals gezegd kunnen zich bij een verlaagde afweer van de patiënt opportunistische infecties voordoen met een ernstig beloop en niet zelden dodelijke afloop.
De lange-termijn bijwerkingen, zoals een veranderde verdeling van het lichaamvet (lipodystrofie), suikerziekte, botveranderingen (osteoporose). Lipodystrofie kenmerkt zich door verlies van onderhuids vet in de armen, benen en het gelaat, alsmede juist een ophoping van vet op de romp en boven de schouders.
Inmiddels is gebleken hoe belangrijk het is de medicijnen trouw en op tijd in te nemen (goede compliantie ['compliance']). Gebeurt dit niet, dan wordt het virus vrij snel verminderd gevoelig voor de medicijnen en ontstaat uiteindelijk resistentie van het virus tegen de voorgeschreven antiretrovirale middelen.
In het Emma Kinderziekenhuis (EKZ) van het Academisch Medisch Centrum in Amsterdam worden sinds 1987 HIV-geïnfecteerde en aan HIV-blootgestelde zuigelingen en kinderen behandeld en gecontroleerd.
Het merendeel van de gegevens in dit proefschrift zijn verzameld binnen het Pediatric Amsterdam Cohort on HIV infection (PEACH). In het cohort worden alle HIV-geïnfecteerde kinderen gevolgd, die de laatste 10 jaar onder behandeling zijn in EKZ/AMC.
Dit proefschrift beschrijft de werkzaamheid (effectiviteit) van HAART om de HIV-replicatie te onderdrukken, om het herstel van de afweer (immuniteit) in de verschillende leeftijdsgroepen te bevorderen, en beschrijft ons onderzoek naar verschillende doseringen van nelfinavir en eventuele factoren die de werking van antiretrovirale therapie bij kinderen kunnen beïnvloeden.
Uit onze beschreven studies blijkt, dat de behandeling van HIV-geïnfecteerde kinderen net zo succesvol kan verlopen als bij HIV-geïnfecteerde volwassenen. De HIV-replicatie kan optimaal onderdrukt worden, al blijkt dit bij jonge kinderen moeilijker te zijn. Ook laten de data zien dat de afweer zich bijna normaliseert t.o.v. gezonde kinderen. Echter, het vermogen om een goede antistofrespons na vaccinatie met BMR en na kinderziektes,

zoals waterpokken, op te bouwen en te behouden is onvoldoende bij HIV-geïnfecteerde kinderen.
Kinderen laten onder HAART een inhaalgroei zien. De meest opvallende lange- termijn bijwerking van HAART is de ontwikkeling van lipodystrofie.
Uit ons onderzoek blijkt dat de antiretrovirale middelen bij kinderen, die volop in ontwikkeling zijn qua groei, per kind moet worden aangepast in verband met een grote variatie tussen kinderen onderling.

Inhoud van dit proefschrift

In Hoofdstuk 2 wordt de lange-termijn follow-up van de behandeling van HIV-geïnfecteerde kinderen met een PI (nelfinavir) en 2 NRTI's beschreven. Na 48, 96, 144 en 240 weken van behandeling met deze HAART combinatie werd HIV bij 74, 66, 58 en 54% van de kinderen succesvol onderdrukt. Hieruit blijkt dat de respons op de behandeling afneemt gedurende een follow-up van 5 jaar. Herstel van de immuniteit verliep even goed bij de kinderen met een goede virale respons, als bij degenen zonder goede respons. Bijna 30% van de kinderen ontwikkelde klinische kenmerken van lipodystrofie.
In Hoofdstuk 3 wordt een studie beschreven, ontworpen om de inname van de medicatie te vereenvoudigen en te onderzoeken of dit de effectiviteit bevordert.
Hierbij werd een combinatietherapie vastgesteld met een gelijk of zelfs betere effectiviteit, waarbij niet van de groep van PI's gebruik zou hoeven worden gemaakt. De NNRTI's is de derde groep HIV-geneesmiddelen, ook wel non-nucleoside reverse transcripase remmers genoemd. Met de keuze van dit regiem hoeven de kinderen de medicijnen slechts 1 maal per dag in te nemen. Deze behandeling omvat 1 NNRTI (efavirenz), in plaats van een PI zoals nelfinavir, in combinatie met 3 NRTI's. Na 48 en 96 weken therapie was bij 76% en 67% van de kinderen HIV optimaal onderdrukt. Bij kinderen, die bij een eerdere behandeling resistenties hadden ontwikkeld, bleek de behandeling net zo succesvol te zijn evenals het herstel van de afweer.
In Hoofdstuk 4 wordt specifiek de afweerrespons op HAART beschreven tijdens de behandeling van 2 regiems met PI en NRTIs bij HIV-geïnfecteerde kinderen in meerdere centra in Nederland. Hieruit bleek dat het herstel van de afweer, m.a.w. het normaliseren van de $CD4^+$ T cellen, onafhankelijk van de leeftijd is. Dit geeft aan dat kinderen op elke leeftijd in staat zijn voldoende $CD4^+$ T cellen te produceren om aan de vraag te voldoen. In het algemeen herstellen kinderen het aantal $CD4^+$ T cellen beter en sneller dan volwassenen, zelfs in een vergevorderd stadium van de ziekte.
In Hoofdstuk 5 wordt de response na vaccinatie met BMR en waterpokken (varicella zoster virus [VZV]) beschreven bij HIV-geïnfecteerde kinderen. Specifieke antistoffen tegen BMR daalden tijdens HAART en verdwenen volledig bij 40% van de kinderen, die BMR-seropositief waren bij de start van HAART. Tevens verloor 21% van de kinderen beschermende antistoffen tegen VZV na een doorgemaakte waterpokken in het verleden (wild-type) en 7% tegen CMV (cytomegalovirus), binnen een periode van 3 jaar op HAART.

EBV (Epstein Barr virus)-specifieke antistoffen bleven echter goed aantoonbaar. De klinische relevantie hiervan is nog onduidelijk, maar het regelmatig controleren van specifieke antistoffen en zonodig hervaccineren is aan te raden.
In Hoofdstuk 6 wordt een studie beschreven, waarbij gekeken is naar HIV RNA. Voor start HAART is een evenwicht tussen aanmaak en afbraak. Na start HAART volgt er een rappe daling. Met name jonge kinderen hebben vaker een hogere concentratie van HIV RNA (HIV virale load) in hun bloed. Wellicht dat hierdoor de behandeling bij kinderen minder effectief is. Uit onze data is gebleken, dat er een relatie is tussen HIV RNA concentratie en leeftijd, maar niet tussen leeftijd en de snelheid van afname van het HIV RNA na start HAART. Waarom hebben kinderen een hogere HIV RNA concentratie? De hoge load is niet noodzakelijkerwijs ten gevolge van een snellere virus-productie. Wellicht ligt de oorzaak bij een nog onrijp immuunsysteem, waardoor misschien andere cellen ook vatbaar zijn voor HIV hetgeen in een grotere pool cellen voor meer virusreplicatie (per lichaamseenheid). Het zou ook kunnen, dat de verhouding tussen het bloed en/of plasmacompartiment, waarin HIV RNA gemeten wordt en het buiten de bloedvaten gelegen lymfeklierstelsel waar het virus zich vermenigvuldigt (o.a. in het maagdarmkanaal), bij kinderen anders is dan bij volwassenen. Helaas zijn deze mogelijkheden om technische en ethische redenen niet eenvoudig te onderzoeken.
In de hoofdstukken 7 en 8 wordt de farmacokinetiek van nelfinavir in een dosering van 30 mg/kg elke 8 uur en een dosering van 45 mg/kg elke 12 uur bij HIV-geïnfecteerde kinderen. Nadat de kinderen gedurende 2 weken waren gestart met HAART werden ze op de dagbehandeling opgenomen en werd via een infuus vlak voor inname van de HAART en op regelmatige tijdstippen gedurende de dag en de ochtend erna voor de volgende inname via een infuus bloed afgenomen voor de bepaling van de concentratie van nelfinavir (dagcurve). De spiegels (plasmaconcentraties), die bij deze doseringen gemeten werden, waren hoger dan bij volwassen personen. Echter vanwege een grote inter-individuele variatie, wordt geadviseerd voor beide doseringen per patient aanpassing van de dosis van nelfinavir te maken op geleide van de plasmaconcentratie. Dit onderzoek geeft aan dat 2 keer daags nelfinavir doseren goed mogelijk is.
In Hoofdstuk 8 beschrijven we een 12-jarige patiënt met AIDS, die is overleden ten gevolge van leverfalen ten tijde van HAART in combinatie met een nieuw en zeer krachtig antischimmel middel, voriconazol. Wellicht zijn er te hoge plasmaconcentraties ontstaan, omdat sommige middelen van het HAART regiem en de voriconazol van dezelfde enzymen gebruik maken bij de verwerking en afbraak in de lever; dientengevolge is een onherstelbare leverschade ontstaan, waaraan zij overleden is. Bij het voorschrijven van HAART moet veel aandacht besteed worden aan de eventuele interactie met andere geneesmiddelen.

Afsluitende opmerkingen

Het controleren van de plasmaspiegels is één van de middelen om de juiste en noodzakelijke inname van de medicatie te bewaken. Het motiveren van therapietrouw is essentieel voor het welslagen van deze langdurige en levenslange vorm van therapie.

Dat is een kunst, allereerst van de patiënten zelf, waarbij elke vorm van ondersteuning en aanmoediging nuttig kan zijn.
Soms moet deze ondersteuning vrij dwingend zijn en tevens een controle van de inname, alles in het belang van het kind zelf. Deze vorm wordt Directly Observed Therapy (DOT) genoemd. Wijkverpleegkundigen of thuiszorg-organisaties die goed geïnstrueerd zijn over medicatie en inname, zijn daarbij onze directe hulp op afstand. Ten tijde van DOT wordt verondersteld dat therapie-falen tot een absoluut minimum beperkt kan blijven.
Het hangt van vele omstandigheden en factoren af, hoe lang zo'n vorm van observatie en begeleiding volgehouden moet worden.
Allerhande afwegingen bij start en continuering van HAART vinden plaats in onderling en multidisciplinair overleg. Alleen op die manier is een langdurig succes van de therapie te garanderen.
Kennis en kunde gaan hand in hand en dat gaat vooral op voor de kinderen zelf. Vanaf een zekere leeftijd geeft eigen inzicht daarbij een goede motivatie. De juiste motivatie kan door ons gegeven worden nu er met de komst van HAART en een toename van het aantal middelen ook een werkelijk reële toekomst aan deze kinderen geboden kan én moet worden.

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