Paediatric HIV-1 infection in Uganda

Natural history and early antiretroviral treatment response

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I dedicate this thesis to my beloved parents who sacrificed so much and committed themselves to teach, guide and provide so that I could soar above the clouds.
Professor Latimer Kamya Musoke (deceased) and Mrs Rebecca Musoke

To the many HIV infected and affected Ugandan children who have endured so much and yet can smile each day. You challenge us to improve your survival and quality of life so that you can become healthy and productive adults.

Contributors

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Abbreviations

3TC	Lamivudine
ABC	Abacavir
AIDS	Acquired immune deficiency syndrome
ART	Antiretroviral therapy
ARV	Antiretroviral
AZT	Zidovudine
CAP	College of American Pathologists
CBC	Complete blood count
CD4	CD4 positive T cells
CD4%	CD4 positive percentage of T cells
COE	Centre of excellence
d4T	Stavudine
ddI	Didanosine
DAIDS	Division of AIDS
DNA	Deoxyribonucelic acid
DRC	Democratic Republic of Congo
EID	Early infant diagnosis
EFV	Efavirenz
GEE	Generalized estimating equations
HAZ	Height-for-age z score
HIV	Human immune-deficiency virus
HR	Hazard ratio
IF	Immunological failure
ILA	International Leadership Award
IMPAACT	International Maternal, Paediatric, Adolescent AIDS Clinical Trials Group
IQR	Inter-quartile range
IS	Immunological success
K-M	Kaplan-Meier
LPV/r	Lopinavir/ritonavir
MOH	Ministry of Health, Uganda
MTCT	Mother-to-child transmission of HIV
MU-JHU Collaboration	Makerere University-Johns Hopkins University Research
NIH	National Institutes of Health, USA
NRTI	Nucleoside reverse transcriptase inhibitors
NNRTI	Non-nucleoside reverse transcriptase inhibitors
NUFU	Norwegian council of universities program for development research and education
NVP	Nevirapine
p24 Ag	p24 antigen (an HIV protein)
PCR	Polymerase chain reaction
RNA	Ribonucleic acid

ROC	Receiver operator curve
SD	Standard deviation
sdNVP	single dose nevirapine
PEPFAR	Presidents Emergency Plan for AIDS Relief
PI	Protease inhibitor
PIDC	Paediatric Infectious Diseases Clinic (HIV clinic-Mulago)
PLH	People living with HIV
PMTCT	Prevention of mother-to-child transmission of HIV
POC	Point of care test
TAM	Thymidine analogue mutations
TLC	Total lymphocyte count
UNAIDS	Joint United Nations Program for HIV and AIDS
UNICEF	United Nations International Children's Emergency Fund
VF	Virological failure
VS	Virological success
WAZ	Weight-for-age z score
WHO	World Health Organization

Abstract

Paediatric HIV-1 infection remains a major problem for sub-Saharan Africa with over 1300 new infections daily. Infected children have a high mortality with 50% dying by 2 years of age. Access to antiretroviral therapy (ART) has significantly reduced the morbidity and mortality rates, however, appropriate monitoring of response to treatment remains a challenge.

Objectives The studies included in this thesis were designed to: 1) describe the natural history of a cohort of HIV infected Ugandan children; 2) determine if the Total Lymphocyte Count (TLC) could be used as a surrogate marker for death in infected children; 3) document the growth, immune and virological response to antiretroviral therapy; and to: 4) document the antiretroviral treatment outcome of children exposed and not exposed to single dose nevirapine (sdNVP) at birth.

Methods Cohort 1 (n=128) included infected children from the perinatal HIV prevention trial (HIVNET012) who were followed from birth to 5 years of age. History and physical examinations were conducted at birth, 6, 10 and 14 weeks, at 6, 12 and 18 months and every following 6 months. CD4% and HIV-1 RNA were measured at birth, 14 weeks, 12 months, and every 6 months thereafter (Papers I & II). Cohort 2 (n= 124) included HIV-1 infected children who were initiated on ART and followed for a minimum of 48 weeks. CD4% and HIV-1 RNA were measured at baseline and 12, 24, 36 and 48 weeks after starting ART (Paper III). Children were classified into virological (V) and immunological (I) treatment success(S) or failure (F) (Paper III). A subset of children (n=92) exposed to NVP (at birth) at a median of 1.7 years prior to ART and not exposed to NVP were initiated on NVP-based ART regimen and followed for 48 weeks (Paper IV). The data was analyzed to determine factors associated with survival and treatment outcome.

Results The infected children had a very high mortality rate with 56/128 (42%) and 70/128 (55%) dying by 2 and 5 years of age, respectively. Time to death was not statistically different for those infected *in utero* compared to those infected later when estimated from time of HIV acquisition. The leading causes of death were pneumonia, diarrhoea and malaria, similar to the uninfected children. There was a rapid decline in CD4% in the first 6 months of life and then a plateau but the HIV-1 RNA levels remained high (5.5 log10) throughout the 5 year follow-up (Paper I). The TLC did not correlate with the CD4 cell count ($r = 0.01$) or predict death in the next 12 months, when the WHO TLC thresholds were used (Paper II). Over 85 % (107/ 124) of the children who initiated ART had an undetectable viral load after 48 weeks of therapy. Those who initiated ART below 3 years of age had a greater increase in weight and height and those children with CD4 % > 15% at ART initiation were more likely to achieve treatment success(Paper III). Baseline CD4 %, age and WHO stage were the baseline factors associated with successful treatment outcome (Paper III). In the sub-study (n=92), after 48 weeks on ART, 75% (33 /44) and 78% (35/44) of the children

exposed and not exposed to sdNVP had an undetectable viral load (< 400 copies/ml), respectively (p=0.8, Paper IV).

Conclusion From the studies included in this thesis, we established that HIV infected Ugandan children had a very high mortality rate in the first 2 years of life, regardless of time of HIV acquisition. The TLC was not an appropriate surrogate marker for death and therefore could not be used to determine which children needed ART. Early initiation of ART was critical to ensure adequate growth and ARV treatment success. Older children exposed to sdNVP at birth benefited from a NVP based regimen and should not be denied access to ART when protease inhibitors (PI) are unavailable.

Original papers

The thesis is based on the following papers:

I
Musoke PM, Mwatha T, Brown E, Bagenda D, Owor M, Lubega IR, Ndugwa CM, Fowler MG, Jackson JB, Guay LA. Natural history of HIV infection in perinatally infected Ugandan children from birth to five years: the HIVNET012 cohort. *Manuscript for submission*

II
Musoke PM, Young AM, Owor MA, Lubega IR, Brown ER, Mmiro FA, Mofenson LM, Jackson JB, Fowler MG, Guay LA.Total lymphocyte count: not a surrogate marker for risk of death in HIV infected Ugandan children. *J AIDS* 2008;**49**:171-8

III
Musoke PM, Mudiope P, Barlow-Mosha LN, Ajuna P, Bagenda D, Mubiru MM, Tylleskar T, Fowler MG.Growth, immune and viral responses in HIV infected Ugandan children receiving highly active antiretroviral therapy: a prospective cohort study. *BMC Pediatr* 2010;**10**:56.

IV
Musoke PM, Barlow-Mosha L, Bagenda D, Mudiope P, Mubiru M, Ajuna P, Tumwine JK, Fowler MG. Response to antiretroviral therapy in HIV infected Ugandan children exposed and not exposed to single dose Nevirapine at birth. *J AIDS* 2009;**52**:560-8.

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Introduction

There are an estimated 2 million children worldwide living with the human immunodeficiency virus -1 (HIV-1) and 90% reside in sub-Saharan Africa [1]. Mother-to-child transmission of HIV (MTCT) remains the major mode of acquisition of HIV-1 infection in children with 400,000 new infections each year [1]. In resource-limited settings infected children have an extremely high mortality with 50% dying by 2 years of age [2]. These children die from common childhood infections including pneumonia, malaria and diarrhoea. Over the last 5 years there has been increasing access to antiretroviral therapy (ART) for people living with HIV/AIDS in low-income countries. However, < 10% of the 3 million people on ART world wide are children [1]. Access to antiretroviral treatment for children lags behind adults because of the limited paediatric antiretroviral drug formulations, complexity of prescribing antiretroviral therapy (ART) in children and an inadequate number of health care workers trained to provide paediatric ART services [3].

Uganda has ~ 1 million people living with HIV-1, 150,000 of whom are children [1]. Without access to prevention of mother to child HIV transmission (PMTCT) services, an estimated 20,000 new infections occur through MTCT each year in Uganda [4]. Similar to HIV-1 infected children from other resource-limited settings, Ugandan infected children have a very high mortality [5]. Using the current WHO recommendations for ART initiation, about 76,750 children are eligible for ART [4, 6]. Currently, 19,954 children are on ART which accounts for only 26 % of the children who require ART and 8 % of all individuals on ART in Uganda [4]. The number of children on ART in Uganda falls below the WHO recommended target of 10% of the total population on ART. This thesis presents the natural history of HIV-1 infected children from the perinatal HIV prevention trial HIVNET012, the utility of the TLC for predicting death in infected children and the early response to antiretroviral therapy (ART) in a cohort of HIV-1 infected Ugandan children.

Natural history of HIV-1 infection in African children

The high HIV-1 seroprevalence in pregnant women from sub-Saharan Africa continues to drive the paediatric HIV-1 epidemic (1). Prior to the introduction of PMTCT services 21-43 % of HIV-1 infected women in Africa transmitted HIV-1 infection to their infants compared to 14-25% in high-income countries [7, 8]. Advanced maternal disease and almost universal breastfeeding contribute to the higher vertical transmission rates in Africa [7]. Despite significant breakthroughs in PMTCT research, along with WHO and country-specific guidelines for implementation of PMTCT services in antenatal clinics only 50% of the pregnant women worldwide are receiving them [1]. As many pregnant women are unaware of their HIV status, their infants remain at high risk of acquiring HIV-1 infection during pregnancy and lactation.

Without antiretroviral treatment, HIV-1 infected children in resource-limited settings have a mortality rate of 45-59% by 2 years of age, compared with 10-20% in Europe and USA [9-13]. Those infants who quickly progress to AIDS and die before 2 years

of age are considered rapid progressors compared to those children surviving beyond 2 years and progress more slowly to AIDS and death [13]. A bimodal disease pattern has been well documented in paediatric HIV-1 birth cohorts from Europe and United States of America, with those infants infected *in utero* having a more rapid disease progression compared to those infected later [9, 14]. Similar data has been reported from Africa but most of the cohorts only have follow-up for the first 2 years of life. A small Rwandan cohort of infected children followed for 5 years showed a 5-fold higher risk of death for children infected in the first 3 months of life compared to those who acquired the infection later, suggesting that children infected earlier were more likely to be rapid progressors [10]. Of the 54 children who were infected and followed for 5 years, 17%, 28% and 35% developed AIDS symptoms by 1, 2 and 5 years respectively. Among the 28 children who died, only 9 had symptoms of AIDS and the median time of survival after the onset of AIDS was 9 months (IQR 4-21 months). In a Malawi cohort of infected children, 35% died by 2 years of age and 45% and 20% were in CDC clinical category B and C, respectively. By 3 years of age, 89% had died and the 10% who were alive were in CDC clinical category B or C, with only 1% asymptomatic [15]. In a report from West Africa, infants who became HIV infected before 12 days and between 13 and 45 days of age had a higher risk of dying compared to those infected after 45 days, with relative hazards of 18.0 (CI 4.8-69) and 7.6 (CI 2.0 -29.5), respectively [11]. In contrast, in the European collaborative study 15% of the children progressed to CDC category C or death by 1 year of age, 40% by 5 years and 50% by 10 years. Those children born after 1995, had a significantly reduced mortality rate of 5% at 12 months of age because of access to ART [16].

Prior to use of antiretroviral therapy, the median survival of infected children from Europe and USA was 8 years compared to 2 years in resource-limited settings [2]. The high numbers and early mortality in infected children from resource-limited settings is due to severe recurrent infections, malnutrition, host immunity, HIV-1 subtype, poverty and limited access to quality health-care [17-20]. Co-infections are associated with immune activation and stimulation of CD4 cells which leads to increased replication of HIV-1, increase in viral load and disease progression [21, 22]. Like previous reports from the USA, African children with a higher viral load (> 250,000 copies/ml) and lower CD4 cell count (< 15%) had a higher risk of death [15, 23]. Maternal factors including high viral load, low CD4 cell count and death also contribute to the mortality in HIV-1 infected children [13].

HIV-1 infected African children have a 10-fold increased childhood mortality risk compared to uninfected children [24]. The causes of morbidity and mortality in the infected children are similar to those children who are uninfected including, pneumonia, malaria and diarrhoea. The HIV-1 infected child tends to have more severe infections, with a slower recovery and response to treatment [25]. The African child has multiple inter-current infections, underlying malnutrition and micronutrient deficiencies that also contribute to the high mortality [26].

Various cohorts of infected children from African countries have demonstrated a high mortality in the first 2 years of life. A cohort study in Kenya found the 2-year

mortality rate from a randomized controlled trial of breastfed vs. formula-fed infants was similar, at 46% and 40%, respectively [27]. The formula-fed infants had a significantly reduced HIV-1 transmission rate, but had a high mortality rate mainly due to diarrhoeal disease and malnutrition. In Uganda, a rural community cohort of HIV-1 exposed children followed for 2 years reported a mortality rate of 54.7% in HIV-1 infected children compared to 16.6% in uninfected children [28]. In West Africa, there was a child mortality rate of 51% at 18 months in a cohort of infected children whose mothers had been randomized to zidovudine or placebo for PMTCT. The diagnosis of HIV-1 infection before 12 and between 13 and 45 days, clinical AIDS, maternal viral load, maternal CD4 cell count and survival were risk factors for child death [11]. In contrast, a study from Durban, South Africa, reported a 2-year mortality rate of 19% in HIV-1 infected children who were exclusively breastfed. This reduced 2-year mortality rate noted in the Durban cohort compared to the other cohorts of infected African children may be related to access to quality health care services, regional differences in the child mortality rate, the higher rate of exclusive breastfeeding and HIV-1 subtype [2, 29].

Table 1. Mortality in HIV-1 infected children from African countries

Country	Predominant subtype in country	Study design	Infected children (n)	Age of mortality estimation	Mortality
Rwanda (Spira R [10])	A & D	Observational	94	2 years	45%
Kenya (Nduati R [27])	A & D	Clinical trial Randomized BF vs. FF	197	2 years	46%
Uganda (Brahmbhatt [28])	A & D	Observational	69	2 years	54%
South Africa (Bobat R [29])	C	Observational BF vs. FF	43	2 years	39%
Malawi, Zambia Tanzania* (Chilongozi[30])	C A & D*	Observation cohort HPTN 024	404	12 months	42%
West Africa Cote d'Ivoire & Burkina Faso (Dabis F[11])	A & G CRF02_AG	Clinical trial Randomized AZT vs. placebo	100	18 months	51%

The high mortality rate of HIV-1 infected children from sub-Saharan Africa is similar across the continent, albeit slightly lower in South Africa (Table 1). The risk factors for early infant mortality include early acquisition of HIV-1 infection, progression to clinical AIDS and maternal health and survival [2, 11, 13]. Without access to antiretroviral therapy this high and early mortality rate of HIV-1 infected children will persist in resource-constrained settings. The scale-up of PMTCT is critical for prevention of new paediatric infections, but also provides an opportunity to identify

HIV-1 infected infants early and refer them to appropriate care and treatment services [2].

Clinical features and diagnosis of paediatric HIV-1 infection

The clinical features of AIDS in both adults and children are related to the severe immune deficiency associated with HIV-1 infection. Children present with recurrent and severe common childhood bacterial infections including pneumonia, otitis media, sinusitis, septicaemia and meningitis. In addition, lymphadenopathy, hepatomegaly, splenomegaly and malnutrition that presents with failure to thrive, stunting and severe acute malnutrition are also common features of infected children from resource-limited settings [2, 17]. In sub-Saharan Africa, 40–50% of children admitted to hospital with severe acute malnutrition are HIV-1 infected [31]. Co-infection with tuberculosis is common in HIV-1 infected children in Africa because of increased exposure to TB from HIV-1 co-infected adults in the home and the higher risk of disease progression after acquisition of primary TB infection [32]. As HIV-1 infection progresses to AIDS the children develop other opportunistic infections and conditions including *Pneumocystis jorveci* pneumonia, cryptococcal meningitis in older children, herpetic and cytomegalovirus infections and HIV-1 associated cancers e.g. kaposi sarcoma. Therefore a child presenting with signs and symptoms suggestive of HIV-1 infection including recurrent bacterial infections, tuberculosis and/or severe acute malnutrition should be tested for HIV-1 to determine its HIV status and ensure appropriate referral for care and treatment. With increased access to early ART, many of these clinical features are becoming less prevalent in infected children.

All infants born to HIV-1 infected women passively acquire maternal IgG antibodies across the placenta during the third trimester of pregnancy, and therefore test positive using routine HIV-1 antibody tests. In the majority of HIV-1 exposed but uninfected infants, the maternal HIV-1 antibodies decay by 9-12 months of age. All uninfected children will lose maternal antibodies by 18 months of age and therefore will test HIV-1 negative using routine rapid antibody tests [33]. Infected infants remain HIV-1 antibody positive after 18 months of age, as they develop their own viral-specific HIV antibodies. Because all HIV-1 exposed infants are antibody positive, the diagnosis of HIV-1 infection in infancy requires viral-specific tests such as ultra sensitive HIV-1 p24 antigen, HIV-1 DNA PCR or RNA PCR [34, 35]. The diagnosis of HIV-1 in infancy remains a challenge in resource-limited settings where access to these viral tests is limited. However, many countries in sub-Saharan Africa are setting up early infant diagnosis (EID) programs using dried blood spots for HIV-1 DNA PCR testing at reference laboratories [33, 35].

Classification of HIV-1 infection in both adults and children has relied on clinical features, specific opportunistic infections and CD4 cell count. The CDC clinical criteria classifies children into 3 groups, A-asymptomatic, B-symptomatic and C-symptomatic with AIDS. However, the CDC classification system had limitations as many of the listed diseases needed advanced diagnostic technology and some of the conditions common in resource-limited settings were not included.

Table 2. WHO Clinical Staging of HIV for infants and children with established HIV-1 infection.[40]

Clinical Stage 1	Clinical Stage 4
- Asymptomatic - Persistent generalized lymphadenopathy	- Unexplained severe wasting, stunting or severe malnutrition not responding to standard therapy - Pneumocystis pneumonia - Recurrent severe bacterial infections (e.g. empyema, pyomyositis, bone or joint infection, meningitis, but excluding pneumonia) - Chronic herpes simplex infection; (orolabial or cutaneous of more than one month's duration, or visceral at any site) - Extrapulmonary TB - Kaposi sarcoma - Oesophageal candidiasis (or candiadisis of trachea, bronchi or lungs) -Central nervous system toxoplasmosis (after the neonatal period) - HIV encephalopathy - Cytomegalovirus (CMV) infection; retinitis or CMV infection affecting another organ, with onset at age more than one month - Extrapulmonary cryptococcosis including meningitis - Disseminated endemic mycosis (extrapulmonary histoplasmosisi, coccidioidomycosis, penicillosis) - Chronic cryptosporidiosis (with diarrhoea) - Chronic isosporiasis - Disseminated non-tuberculous mycobacterial infection - Cerebral or B-cell non-Hodgkin lymphoma - Progressive multifocal leukoencephalopathy - HIV-associated cardiomyopathy or nephropathy
Clinical Stage 2	
- Unexplained persistent hepatosplenomegaly - Papular pruritic eruptions - Extensive wart virus infection - Extensive molluscum contagiosum - Recurrent oral ulcerations - Unexplained persistent parotid enlargement - Lineal gingival erythema - Herpes zoster - Recurrent or chronic upper respiratory tract infections (otitis media, otorrhoea, sinusitis, tonsillitis) - Fungal nail infections	
Clinical Stage 3	
- Unexplained persistent hepatosplenomegaly - Papular pruritic eruptions - Extensive wart virus infection - Extensive molluscum contagiosum - Recurrent oral ulcerations - Unexplained persistent parotid enlargement - Lineal gingival erythema - Herpes zoster - Recurrent or chronic upper respiratory tract infections (otitis media, otorrhoea, sinusitis, tonsillitis) - Fungal nail infections	

The WHO paediatric clinical staging developed for resource-limited settings consists of stage I - asymptomatic, stage II –mild symptoms, stage III- moderate symptoms and stage IV-severe symptoms with AIDS (Table 2). In adults and children > 5 years of age, absolute CD4 cell count is used to monitor disease progression and level of immune suppression (Table 3). In children < 5 years of age CD4% is more reliable for assessing the degree of immune suppression. All infants are born with a high absolute CD4 cell count, regardless of HIV-1 status, experience a steady decline in CD4 cell count with age, until about 6 years of age when they reach adult levels [36, 37]. Therefore, infected children < five years of age are monitored using CD4% which is less age dependent and more reliable to determine degree of immune suppression (Table 3).

Table 3.WHO immunological classification for established infection in infants and children.[40]

HIV-associated immunodeficiency	<11 months (CD4%)	12-35 months (CD4%)	36-59 months (CD4%)	>5 years (CD4 count)
None or not significant	>35	>30	>25	>500
Mild	30-35	25-30	20-25	350-499
Advanced	25-29	20-24	15-19	200-349
Severe	<25	<20	<15	<200 or <15%

The CD4 cell count and viral load (HIV-1 RNA) are surrogate markers for disease progression in children and adults [6]. Peak and set-point plasma viral levels in infants are considerably higher than those in adults [38]. This difference in peak viraemia is most likely due to the immature immune system in infancy which poorly contains HIV-1 replication leading to higher levels of circulating virus and a more rapid disease progression in children [39]. Viral load testing is not readily accessible in most centres in Africa, and therefore weight and CD4% are used to monitor response to ART in most children in resource-limited settings [6].

Table 4. The immunological cut-offs for initiation of ART in children according to WHO Antiretroviral therapy guidelines 2006.[41]

Immune marker	Age-specific recommendation to initiate ART			
	<12 months	12 - 35 months	36 - 59 months	≥5 years
CD4 percent	All	<20%	<20%	<15%
CD4 count/mm^3	All	<750 cells	<350 cells	<200 cells
TLC/mm^3	All	<3000 cells	<2500 cells	<2000 cells

Clinical criteria for ART – WHO clinical stage 3 and 4

Identifying infected children at highest risk of disease progression and death is difficult in infancy because the CD4 cell count and HIV-1 RNA do not predict death [13]. In older children, the CD4 cell count remains the stronger predictor for death to determine which children are at highest risk and require ART. The CD4% is the main indicator for immune function that is used to determine which children require ART. The WHO developed age-appropriate CD4% cut-offs to guide health-workers in resource-limited settings about when to initiate ART (Table 4).

Antiretroviral therapy in resource-limited settings

Access to antiretroviral therapy has made a significant difference in the lives of infected adults and children with decreased morbidity and mortality [3, 42, 43]. The improved survival and quality of life of children on treatment has been evident in high-income countries since effective ART became available [44]. However, there remains a large gap in resource-constrained countries between the number of infected individuals that required ART and those who receive it. Currently only 42% of the 2,700,000 HIV infected people in sub-Saharan Africa needing ART receive it, < 10% being children [1]. Only 37% and 28% of infected adults and children, respectively, who are eligible for ART are receiving treatment [45].

The initial costs of effective ART made the treatment prohibitive for the majority of infected people in resource-limited countries. In 2003, WHO set a target to treat 3 million people from resource-limited settings with antiretroviral therapy by 2005 [46]. With support from Global Fund for AIDS, TB and Malaria, and the President's Emergency Plan for AIDS Relief (PEPFAR), low-income countries started national treatment programs and finally made access to ART a reality in Africa. Recognizing that children were grossly underserved, UNICEF and its partners launched the global campaign 'Unite for children, unite against AIDS' in 2005, in an effort to increase HIV diagnosis and treatment of infected children and improve linkage to the already existing PMTCT programs [47].

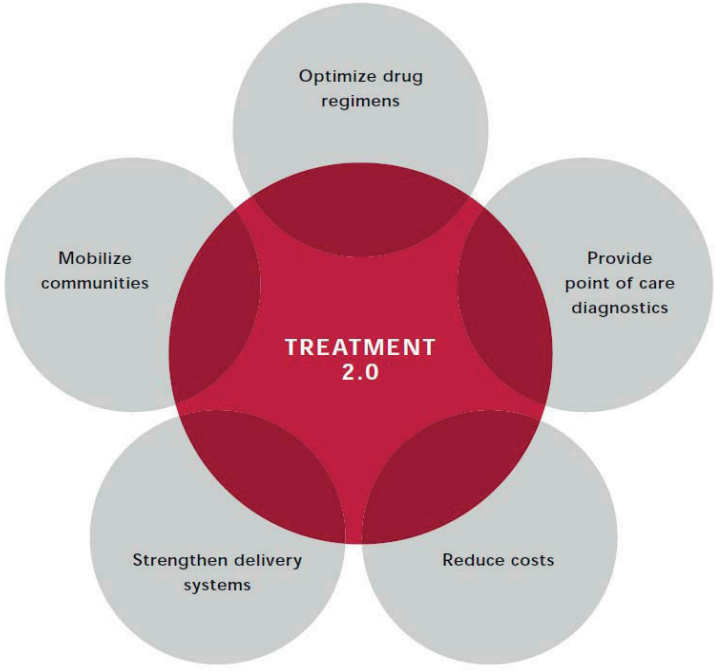

Figure 1. UNAIDS schema for optimizing antiretroviral therapy (UNAIDS Global HIV Report 2010).

Despite increased access to ART in resource-limited settings the coverage for children has lagged behind that of adults [45]. However, there have been some success stories where children initiated on ART in Africa have shown similar immunological and virological responses as their counterparts in Europe and USA [3, 48-51]. The challenges of providing ART for children include the lack of appropriate paediatric drug formulations, limited ARV drug choices, the need to adjust the drug doses according to a child's weight, the inadequate number of well trained health-workers to prescribe and monitor therapy, as well as the overall complexity of delivering paediatric care and treatment. UNAIDS recognizes the complexity of delivering ART in resource-constrained settings and recommends the strengthening of the 5 pillars of treatment to improve services and treatment outcomes (Figure 1).

Antiretroviral therapy in African children

At the end of 2009, ~ 1.27 million children in sub-Saharan Africa were in need of ART, but only 354,000 were receiving ART, a coverage of 28% [1]. Most of the children initiated on antiretroviral treatment were over 5 years of age and had survived early childhood without ART [3, 51-53]. The majority of children were initiated on the WHO recommended first-line regimen of 2 nucleoside reverse transcriptase inhibitors (NRTIs), stavudine (d4T) or zidovudine (AZT) plus lamivudine (3TC), and one non-nucleoside reverse transcriptase inhibitor (NNRTI), nevirapine (NVP) or efavirenz (EFV), as demonstrated by the UNAIDS survey from 36 countries (Figure 2). Initially, only adult fixed-dose ARV drug combinations were available and were also used for infected children with successful treatment outcomes [54, 55].

A review of over 30 cohorts of HIV infected children from Africa who were initiated on ART with a range of 50 to 4000 children per study was reported in 2008 [3]. At ART initiation the median age was 5 years and the median CD4 percent was 6 -15%. The majority of children were malnourished with weight for age (WAZ) and height for age (HAZ) scores less than -2 SD below normal. Mortality was < 10% in those children followed for 12 months with the majority of deaths occurring in the first 6 months on therapy.

Eleven centres used adult fixed dose combinations and the children were dosed according to weight bands. Over half of the studies documented an improvement in nutritional and clinical status with an average weight gain of 1.8–3.6 kg in the first year of treatment. The median CD4% increased significantly in the first year with a plateau after 12–18 months and the viral load decreased by ~ 2.0 log10 copies per ml within 1 year of starting therapy. The large number of children with malnutrition, older median age and very low CD4% at ART initiation has been documented in multiple paediatric treatment programs from Africa [3, 49, 53, 56]. With the scale-up of PMTCT, early infant diagnosis (EID) and the WHO recommendation to initiate ART in all infected children < 2 years of age, there is an opportunity for younger and more immune competent children to be initiated on ART [57].

Monitoring the response to antiretroviral therapy in resource-limited settings remains a challenge because laboratories that perform CD4 cell counts and HIV-1 RNA

measurements are limited to a few urban centres. Treatment programs in most African settings use weight and occasionally height with CD4%/counts where available, to monitor the response to ART. Children initiated on ART can achieve adequate growth and immune responses regardless of incomplete viral suppression [58, 59]. The children achieve normal weight for age z score within 6–12 months of ART but gain in height is often delayed [58]. It is difficult using only clinical monitoring to detect the emergence of resistant mutations and virological failure, which limits future treatment options. Therefore, low-cost point of care tests for CD4 cell count estimation and HIV-1 RNA measurements are urgently needed in Africa.

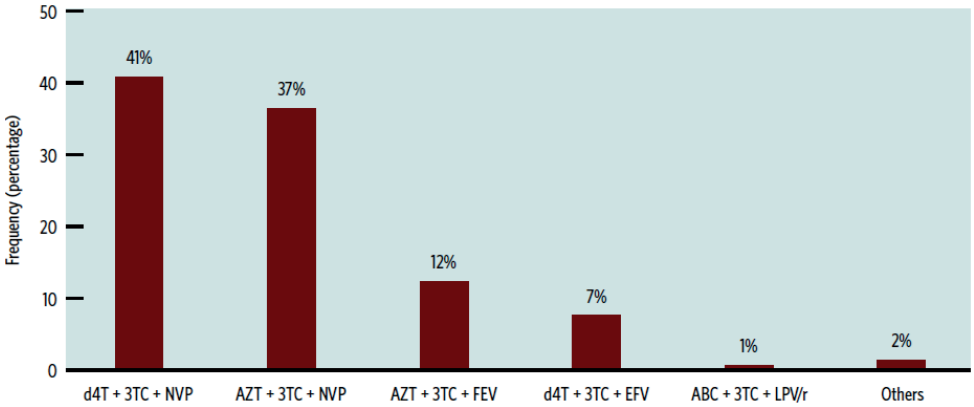

Figure 2. First line regimen in children from 36 low and middle income countries (n =177,064) December 2008 (UNAIDS scaling up ART)

Adherence is the cornerstone to successful ART and requires the child to take >95% of the prescribed ARV drugs [60, 61]. The emergence of resistant mutations when children on ART fail to completely suppress virus is well documented [51, 62, 63]. For children on ART for >48 weeks, HIV-1 resistant mutations have been reported in 20–50 % of those children who fail to completely suppress virus. The most common resistance mutations identified through genotypic analysis, usually detected within 1-3 months after failure, include M184V, which confers resistance to lamivudine and Y181and K103N confers resistance to NVP and EFV [64]. The T215F mutation for AZT tends to emerge later and may not be detected before 12 months of failure [65].

The longer children are maintained on a failing ART regimen the greater number of thymidine analogue mutations (TAMs) to emerge with an increased cross-class resistance [66, 67]. As more infants are exposed to NVP in infancy and other children fail their first-line ART regime, many more children will develop resistant mutations requiring a treatment switch. Second line ARV drugs are more expensive, tend to be more difficult to administer and have a higher pill burden. Therefore, it is critical that children and families are provided with ongoing counselling and support to enable infected children to maintain adequate viral suppression for as long as possible on their first-line regimen, in order delay the need to switch to second line therapy.

Response to NNRTI-based ART after nevirapine exposure at birth

With the scale-up of PMTCT, most HIV-1 infected infants who fail ART prophylaxis will have prior exposure to NVP with emergence of NNRTI resistant mutations, which do fade over time. [68]. Subsequent therapy with an NNRTI based regimen in infants exposed to sdNVP has been associated with higher rates of virological failure when compared to those infants not exposed to nevirapine (NVP) [69-71]. Therefore, WHO recommends that all infected infants exposed to NVP during the peripartum period, or during infancy, should be initiated on a protease inhibitor (PI) based regimen for prevention of breast milk transmission [6]. However, the scale-up of PI based regimens for these infants remains a challenge in many resource-limited countries because the accessible PI, lopinavir/ritonavir (LPV/r), is more expensive than the NRTIs and NNRTIs. In addition, the syrup formulation requires refrigeration and a tablet cannot be divided in half, making it difficult to administer to young infants.

In a multi-centre randomized controlled trial of a PI vs. NNRTI regimen in children exposed to NVP at birth (P1060), the children in the NNRTI arm had a higher virological failure rate (44% vs. 20%) compared to the children in the PI arm. However, the median age was 0.7 years, making it impossible to determine if this virological failure rate would have been lower in older children if they had been followed for a longer period than the study endpoint of 24 weeks after ART initiation [70]. Of note, 60% of the children in the NNRTI arm did achieve complete viral suppression despite prior exposure to sdNVP. Therefore, where PIs are not accessible infected children may still benefit from a NVP-containing ART regimen and should not therefore be denied ART. These recent study results are important for guiding recommendations for 1st line ART in Africa where many infected children will have had prior NVP exposure at birth as part of PMTCT.

Paediatric antiretroviral therapy in Uganda

Access to free ART for children started in 2002/3 through donations from different philanthropic organizations. However, in 2006, through PEPFAR and Global Fund for TB, HIV and Malaria, ART became more widely available in Uganda for both adults and children. Children initiated on ART experienced a significant reduction in morbidity and mortality, with an improvement in their overall quality of life [48, 51, 72].

Of the 150,000 children living with HIV-1, ~75,000 require antiretroviral therapy but only 26% are receiving therapy (1). By 2010, Uganda had 248,200 individuals on ART, with 19,854 (8%) of them children under 15 years of age [73]. Of the 416 ART facilities providing ART in the country, only 296 also provide paediatric ART services. The majority of infected children on ART receive treatment from urban centres, with one third registered at clinics in Kampala, the capital city. The National ART guidelines recommend 2 NRTIs and 1 NNRTI as first line therapy for HIV-1 infected children, except for those infants exposed to NVP, where a PI-based regimen is recommended, consistent with the current WHO ART guidelines for children [6, 74].

The MOH recommends use of fixed-dose combination ARV drugs for children because they are cheaper than syrups, less bulky, easier for the caretaker to administer, and for the health-worker to monitor adherence [74]. The use of fixed-dose combinations for paediatric ART enables a significant reduction in the bulk of drugs dispensed to caregivers (Figure 3). Fixed-dose combination ARVs improve overall adherence and are more acceptable to care givers with young children [75]. However, there are no ARV fixed-dose combinations with LPV/r so that many of the infants on ART, outside the centres of excellence, are still initiated on a NVP based ARV regimen. The MOH is currently updating its national ART guidelines and improving the drug-supply chain management through the national medical stores (NMS). This should provide further guidance to the health-workers delivering ART services and prevent ARV stock outs at health units.

Figure 3. Comparison of a month's supply of first-line ART (A) syrups (B) single tablets and (C) fixed-dose combination tablets [Photographs A & B – ARROW study, Mulago by Bethany Naidoo, Photograph C – MUJHU clinic].

The adherence rate to ART in children from a large HIV clinic in Kampala was much higher than reports from resource-rich settings with > 90 % of the children having

good adherence when using the self report method [60, 76]. However, using an unannounced pill counts method, a much lower levels of adherence was found when compared to self report, 74% vs. 94%, respectively. Children receiving ARV fixed-dose combinations achieve much higher levels of adherence compared to those children on syrup formulations [75]. Many of the children from the paediatric ART programs at centres of excellence (COE) in Uganda receive additional adherence counselling for their HIV infected children and care takers, which contributes to good adherence to ART. In addition, in the family-based comprehensive HIV care program having the mother and the HIV infected family members all attending the same clinic and on the same day enabled similar messages to be shared with the family and for the different members to support each other to adhere to their medication [77]. It is not clear if this high level of adherence is maintained after being on ART for a long time, after improved health of the child and development of treatment fatigue by the caregiver. To improve adherence, there is an urgent need to increase the availability of various fixed-dose combination ARV regimens for children and develop innovative strategies for adolescents.

Between 2004 and 2005 at the national referral hospital, Mulago, a cohort of 250 children were initiated on ART; mean age of 9 years (SD 4.5) and a median CD4% of 8.6% (3.5–12.7%). After 12 months of ART, 164/222 (74%) of the children had an undetectable viral load compared to 392/454 (86%) of the adult population but the children had a more robust immune response. Overall mortality was 28/250 (11%) with over 60% of deaths occurring in the first 3 months on therapy. The predictors of virological failure were CD4 percent < 5%, male child and being on a d4T-based regimen. All 8 samples that had genotype resistance testing done at 12 months documented NNRTI resistance and the 3TC-associated mutation (M184V) [51]. Kiboneka *et al* documented their experience of 770 children initiated on ART and followed for a median duration of 377 days [48]. Similar to other reports from Africa, the children achieved a good clinical and immune response, with a low mortality rate of 2.3%. They noted very high levels of adherence in this cohort with 94% of the children achieving greater than 95% adherence to ART. However, orphans were noted to initiate ART at an older age, have a lower CD4 cell count and a more advanced WHO clinical staging. The adherence levels noted in this study were much higher than in other reports from Africa [60, 78]. Both these programs provided additional adherence counselling to support the children during ARV treatment.

Adherence remains the major challenge to achieving a sustained response to antiretroviral therapy, and requires additional counselling and support for infected children, particularly for orphans who often have multiple care-givers. Significant improvement in appearance and growth has been documented in many children initiated on ART (Figure 4).

Baylor College of Medicine Children's Foundation Uganda (Baylor-Uganda) supports the paediatric HIV clinic at Mulago hospital, Kampala and several satellite clinics countrywide. PEPFAR has provided additional financial support through Baylor Uganda to expand paediatric HIV care and treatment services countrywide. Currently

the foundation has over 6900 infected children registered with 4000 of them on ART. The median age at ART initiation was 8 years in 2006 and has subsequently reduced to 5 years of age. The outcome of paediatric ART is similar across the country with a significant increase in growth and CD cell count. About 70% of the cohort of children who had access to viral load monitoring have an undetectable viral load after 48 weeks of ART (A Kekitiinwa PIDC personal communication). In the Baylor Foundation Uganda supported ART clinics, over 80% of the children who initiated ART are still in follow-up but in contrast adherence to clinic visits across the country is much lower, with only 60% of the children initiated on ART still in follow-up after 60 months [4].

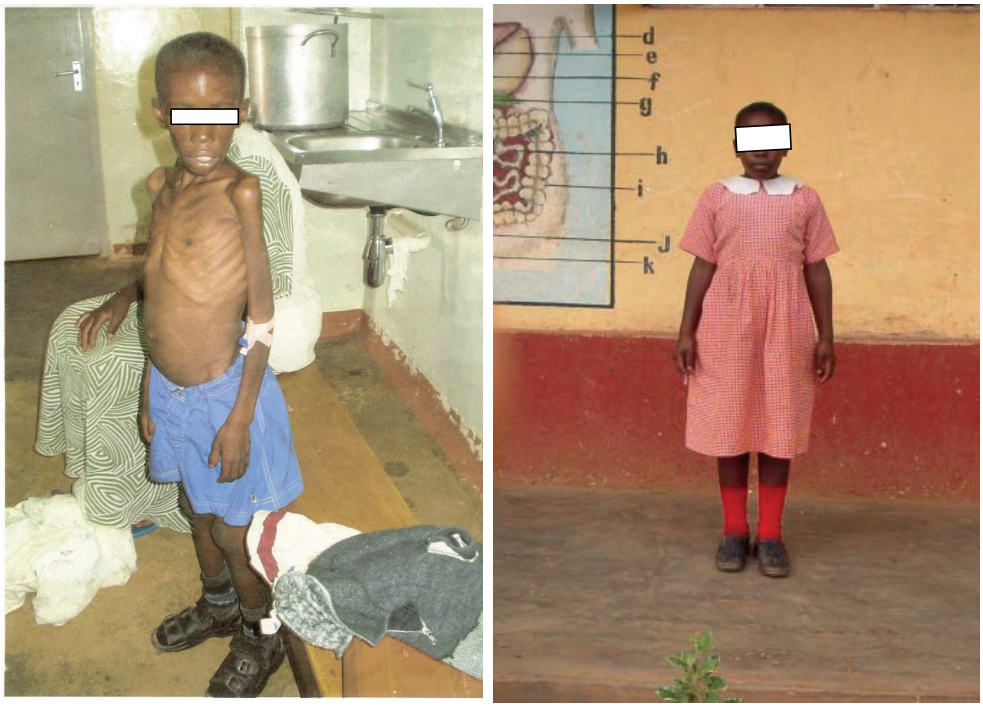

Figure 4. A 12 year HIV-1 infected female child at MU-JHU before ART initiation and after one year of therapy.

Despite the significant improvement in survival and quality of life on ART, the individual ARVs are associated with complications and toxicities. Children tend to experience fewer adverse side effects on ART compared to adults [51]. In Uganda, similar to reports from other African countries where children are on ART, < 5% of the children develop toxicities secondary to ART [50] . The commonly reported side effects include severe anaemia from AZT, hepatotoxicity and a rash from NVP, lipodystrophy from d4T/AZT, and dyslipidaemia usually from PIs. [50, 79]. Due to limited access to laboratory services that can provide routine haematology and chemistries toxicity monitoring, laboratory-based adverse events are only documented in children receiving ART at centres of excellence in Uganda. Children on ART must be closely monitored to ensure good adherence and, adequate clinical and immunological response to ART, in order to delay treatment failure, which limits

future treatment options. Without access to virological monitoring in most resource-limited settings, adherence to the first-line ARV regimen is critical, in order to delay the need for more expensive second line regimens.

Aim and study objectives

Aim

The overall aim of this thesis was to describe the natural history of paediatric HIV-1 infection in Uganda and document the early growth, immune and viral responses to highly active antiretroviral therapy in HIV-1 infected children including those children exposed to single dose nevirapine at birth.

Specific objectives

1. To describe the natural history of a cohort of perinatally HIV-1 infected children from birth to five years of age. (*Paper 1*)

2. To determine the utility of the total lymphocyte count (TLC) as a surrogate marker for risk of death in HIV-1 infected Ugandan children. *(Paper II)*

3. To document the growth response to antiretroviral therapy and determine clinical factors associated with successful antiretroviral treatment outcome. *(Paper III)*

4. To determine the response to a NVP containing ART regimen in children exposed and not exposed to single dose NVP at birth. *(Paper IV)*

Study subjects and methods

Study area

The study was conducted in Uganda, a landlocked country located in East Africa bordered by Kenya on the east, Tanzania in the south, Sudan in the north and the Democratic Republic of Congo (DRC) on the west. It is a beautiful country on the equator covering 240,000 sq km with an average altitude of 1100 m above sea level. The climate is tropical with temperatures ranging between 15-30 °C with two rainy seasons per year.

Uganda has a population of ~32 million people and one of the highest fertility rates of 7 children per woman and an infant mortality rate of 76 per 1000 live births [80]. The national HIV-1 seroprevalence has significantly reduced over the last decade and stands at 6.4 %. Currently there are a million people living with HIV-1 infection of which ~150,000 are children. Without access to PMTCT over 20,000 infants are newly infected with HIV-1 each year.

Figure 5. Map of Uganda within the Arica and East African region.

Kampala is the capital city of Uganda and has a population of ~2 million. Mulago hospital in Kampala is the largest national referral and University teaching hospital with > 1500 beds and multiple outpatient clinics. The paediatric HIV clinic linked to the Baylor College of Medicine and supported by PEPFAR has over **6900** infected children registered for care and treatment and **4000** on antiretroviral therapy. The PMTCT program based in the Mulago hospital antenatal clinics are supported by MU-JHU and over 3000 HIV infected women are identified through the program each year.

The HIV-1 infected and exposed infants are linked to the comprehensive Baylor Uganda paediatric HIV care and treatment clinic.

Study site

Figure 6.The MU-JHU Research Collaboration Site. Top row – external view and bottom row: inside clinic, mother at dispensing window and nurse weighing infant.

The Makerere University – Johns Hopkins University (MU-JHU) research clinic is located in 3 double story buildings on the Mulago hospital complex campus and can refer study patients requiring inpatient services to the hospital (Figure 6). The research collaboration was started by investigators from MU and JHU who wanted to study the natural history of maternal and paediatric HIV-1 infection, and develop strategies to prevent MTCT in resource-limited settings. MU-JHU has been in existence since 1988, involved in multiple perinatal HIV prevention clinical trials including the landmark HIVNET012 study which led to the use of sdNVP for prevention of MTCT in resource-limited settings [81, 82].

In 2000, MU-JHU supported Mulago hospital to set up their PMTCT program, initially using sdNVP, but currently implementing more efficacious ARV regimens. Over the last 8 years MU-JHU has also provided comprehensive HIV care and treatment to women identified through the PMTCT program, as well as their infected partners and children.

Since 2003, the clinic has enrolled and followed >1000 adults and children in a comprehensive family HIV care and treatment program through the MTCT plus of Columbia University, New York [83]. Currently there are 800 participants in active follow-up with 500 on antiretroviral therapy including 150 children. The site also received the International Leadership Award (ILA) funded through Elizabeth Glaser Pediatric AIDS Foundation that enabled children to be initiated on ART, as early as 2004, when there was limited access to ART in Uganda. The data for paper III and IV were from children initiated on ART in the ILA program.

Currently the site is conducting multiple National Institutes of Health (NIH), International Maternal, Paediatric and Adolescent AIDS Clinical Trials (IMPAACT) network studies including P1060 whose primary objective was to determine the response to a NNRTI vs. a PI based regimen in children exposed and not exposed to sdNVP at birth. The majority of MU-JHU study participants live within a 20 km radius of the clinic. For the studies included in this thesis, the participants were enrolled from 1997 to 2004 for the HIVNET012 study, and from 2004 to 2006 for the ILA ART study (Figure 7).

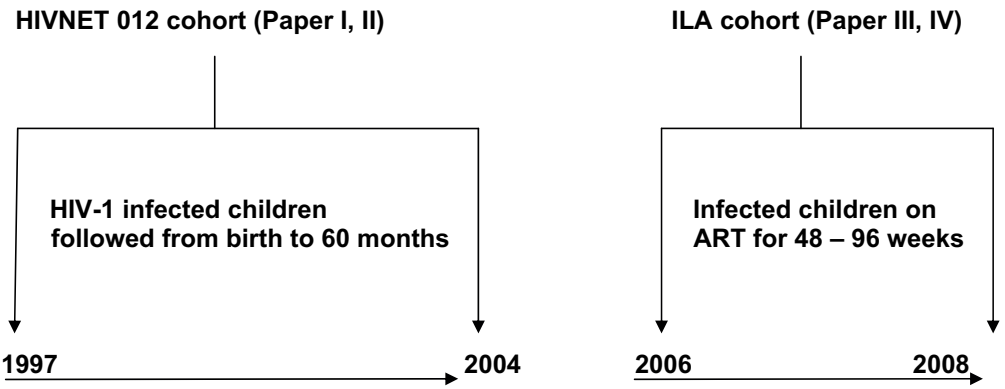

Figure 7. Timeline for the studies included in the thesis.

Methods

All the studies included in this thesis utilized a prospective cohort design. The first cohort of HIV-1 infected children was followed from birth to 5 years of age (Papers I and II) and the second cohort of infected children was initiated on ART and followed for a minimum of 48 weeks (Papers III and IV). The study population, design and analysis for the four papers are summarized in Table 5.

Study population for Paper I and II

The participants for Paper I and II were HIV-1 infected children from an NIH Division of AIDS (DAIDS) perinatal HIV prevention clinical trial (HIVNET012) conducted at the MU-JHU clinic, Mulago Kampala [81, 82]. The trial was a randomized controlled clinical trial in HIV-1 infected women to determine the effectiveness of NVP to the mother at the onset of labour, and to her infant within 72 hours of delivery in preventing mother-to-child HIV transmission. All the participants were enrolled into the study after providing written informed consent.

Table 5. Summary of the study population, design and analysis for the different papers

Paper	Topic	Study population	Study design	Analysis
I	Natural history of HIV infected Ugandan children	HIV-1 infected birth cohort at the MU-JHU clinic, Kampala UG (1997 – 2004) N= 128	Prospective five year cohort study of perinatally HIV infected children	Univariate Cox regression model Mixed effects models for CD4 and HIV-1 RNA Kaplan Meier estimates for survival since HIV acquisition Kaplan Meier estimates for time to event (hospitalization)
II	Use of the total lymphocyte count as a surrogate marker for risk of death	Same as above	Retrospective review of longitudinal TLC and CD4 cell count measurements from a prospective five year cohort of HIV infected children	Partly conditional survival methods for CD4 cell counts, TLC and HIV-1 RNA Cox proportional hazard regression models ROC curves for sensitivity and specificity of TLC compared to WHO CD4 cell cut-offs
III	Growth, immune and viral response to antiretroviral therapy	Cohort of HIV-1 infected children initiating fixed dose combination ART (n=124) 2004 - 2006	Prospective cohort of HIV infected children initiating HAART and followed for 48 weeks	Primary endpoint was viral load <400 copies/ml and CD4% above severe immune suppression. Logistic and multinominal regression, univariate, multivariate GEE to analyze weight and height velocity
IV	Response to NVP based HAART in HIV infected children exposed and not exposed to sdNVP	Same population as III but a sub-study N=94 (2004 – 2006)	Observational prospective cohort of HIV infected children initiating HAART and followed for 48 weeks	Primary endpoint was sustained achievement of viral load <400 copies/ml K-M estimates of conditional probabilities of achieving endpoint at 48 weeks Cox regression analyses indicating hazard ratios associated with various baseline characteristics

There were 654 HIV-1-positive women enrolled in the HIVNET 012 trial, 637 delivered live born infants. Of the 617 delivered infants analysed, 128 (21%) became HIV-1 infected during the follow-up period with 10% testing HIV positive at birth.

The initial study duration was 18 months but it was extended to allow for 5-year follow-up of all infants to monitor long term safety of perinatal exposure to NVP and AZT. This enabled the close follow-up of a cohort of HIV-1 infected and uninfected children from birth until 5 years of age.

Physical exam and laboratory assessment
All infants had a medical history and physical examination at all the scheduled visits including birth and 7 days; 6, 10, 14 weeks;6 months and then every 6 months until 60 months of age. Complete blood counts (CBC) and CD4%/counts were done at birth, at 14 weeks, at 12 and 18 months and then every 6 months for those children who were HIV-1 infected. Qualitative HIV-1 RNA PCR assays were done at age 1-3 days, 6, 14 weeks and 12 months. Quantitative HIV-1 RNA PCR was done at all subsequent visits for those found to be HIV-1 infected at the previous visit.

Statistical analysis

Paper I
For this analysis the HIV-1 infected infants were divided into 2 groups, those who were HIV positive at birth and those who became infected later. The Cox regression model was used to determine association between maternal and infant characteristics and death from time of HIV-1 acquisition. Median CD4 percent and HIV-1 RNA over time from birth to 60 months was analyzed using the mixed effects model and presented as graphs. Kaplan Meier curves were used to estimate survival and time to event (hospitalizations) for the 2 groups.

Paper II
A Cox proportional hazards regression model was used to estimate the risk of death within one year by CD4% and TLC. The models were adjusted for age at the time of the measurement. Probabilities of death within 1 year were calculated by estimating the baseline survival function by non-parametric maximum likelihood method from the predicted survival probabilities. Time-dependent receiver operator curves (ROC) for markers of mortality were estimated to evaluate the ability of CD4% and TLC to identify death within one year from the time of measurements. The sensitivity, specificity, PPV and NPV of the WHO TLC threshold relative to the WHO CD4% threshold for determining severe immune-suppression were calculated according to age groups (<12 months, 12–35 months, and >35–60 months).

Study population Papers III and IV

Study participants for paper III and IV were HIV-1 infected children from the Mulago Paediatric HIV clinic and MU-JHU Research clinic, Kampala, who were eligible for ART according to the WHO antiretroviral therapy guidelines 2002. The children were enrolled into the study after the parents/caretakers provided written informed consent. They were enrolled into a prospective observational study for a period of follow-up of 24-96 weeks. A total of 130 children were enrolled and initiated on ART as part of the International Leadership Award (2003). A subset of them (94) were also enrolled in a study to determine the response to a NVP-based ART regimen in children exposed and

not exposed to single dose NVP at birth. Both cohorts were followed prospectively and after initiation of ART were seen at 2 and 4, weeks, and then every 12 weeks until 96 weeks of age. CD4 cell counts/percents and HIV-1 RNA measurements were done at baseline and every 12 weeks until study end.

Antiretroviral drugs, a fixed-dose combination of d4T, 3TC and NVP were prescribed and dispensed on a monthly basis. All the children had > 48 weeks of follow-up excluding those who died, and 60% of the children had 96 weeks of follow-up.

Statistical analysis

Paper III
The primary endpoint for this analysis was achievement of a viral load of < 400 copies/ml at 24 weeks that was sustained through to 48 weeks, and achievement of CD4 cell count/percent above the level of immune suppression for age. The children were classified into treatment outcome groups: virological and immunological success (VS/IS), virological success and immunological failure (VS/IF), virological failure and immunological success (VF/IS) and both virological and immunological failure (VF/IF). Mean WAZ and HAZ scores at the different time-points of follow-up after ART initiation were compared using Scheffe's multiple comparison test. Factors associated with treatment success were analyzed using logistic regression, and presented as univariate and multivariate analyses. Generalized estimating equations (GEE) were used to analyze weight and height velocity for different age groups during the 48 weeks of follow-up adjusting for various baseline factors.

Multinomial logistic regression was used to determine factors associated with the different treatment outcome groups. All statistical analyses were assessed for statistical significance at the $p < 0.05$ alpha level.

Paper IV
The primary endpoint for this analysis was the sustained achievement of a viral load <400 copies/ml, and the analyses presented with reference to this endpoint are Kaplan-Meier estimates of conditional probabilities of achieving the endpoint through 48 weeks following initiation of HAART. Differences between Kaplan-Meier curves are assessed for statistical significance using the log-rank test. In addition, Cox regression analyses adjusting for, and indicating the hazard associated with the various baseline characteristics measured are presented. All statistical analyses were assessed for statistical significance at the $p <0.05$ alpha level.

Summary of results

Paper 1 and II

HIVNET 012 Study cohort
In the HIVNET 012 study, there were 128 HIV-1 positive children identified, with 52% female. Of these, 42 HIV-1 infected children (33%) died within the first 18 months of life and 17 children died after 2 years of age, a total of 70 deaths over the 5 year follow-up period (55%). Of the 499 HIV-1 negative children, 51% were female, and 26 (5%) and 35 (7%) infants died by 18 months and 5 years of age respectively.

Paper I
Of the 128 infected children 59/128 (46%) were HIV-1 positive at birth (*in utero* transmission) and 69/128 (54%) became HIV infected later (postnatal transmission). There were 75/128 (59%) and 47/128(36%) infants exposed to intra-partum zidovudine and nevirapine, respectively. The total duration of study follow-up was 4,468 child months, with a median follow-up time of 30.4 months (range 1.3-65.2) per child. The median CD4 cell count and HIV-1 RNA at delivery of 299 cells/mm^3 (IQR 170 -507) and 4.8 log copies/ml (IQR 4.5 -5.1), respectively. There were no significant differences in maternal characteristics for the infants in the 2 groups, except for a trend towards a higher median viral load at delivery in infants infected postnatally.

The infants had a median birth weight of 3,025 grams and 10% had a low birth weight (<2500 grams). There were no differences in baseline infant characteristics between those infants who were positive at birth and those who became infected later, except for the duration of breastfeeding, 381 (CI 224-540) vs. 540 (CI 454-724) days, respectively. In the univariate Cox regression model, there were no maternal or infant factors significantly associated with death except maternal viral load at delivery (p= 0.04). When stratified by time of HIV-1 acquisition, the Kaplan Meier estimates for survival from time of infection was similar in the 2 groups of children (p=0.17). The leading causes of death and hospitalization were pneumonia, diarrhoea and malaria.

The median CD4% at birth was 42.6 % (IQR 31.6–51.6) and declined to 24.6% (IQR 18.6–33.6) at 12 months, 19.3% (IQR 13.4-27.0) at 24 months and 20.1% (IQR 14.7-26.1) at 60 months of age (Paper I, Table 3). By 12 and 24 months of age, 50% of the children had CD4% below 25% and 20%, respectively. At 60 months of age only 25% of the children had CD4 percents below 15%. The CD4% decline in both infant groups was similar and median HIV-1 RNA levels remained stable at a median of 5.5 log10 copies/ml for the 60 months of follow-up.

Paper II
In the first 18 months the median TLC in infected children ranged from 4150–5800 cells per µl, which was similar to HIV negative children whose median TLC range was 4200–5600 cells per µl. In contrast, the CD4% of HIV-1 infected children dropped

from a median of 41% to 19% compared to HIV-1 negative children whose median CD4% remained above 38% throughout this period.

The risk of death within one year was assessed using the WHO recommended age-specific TLC threshold values. The risk of mortality was highest for the youngest children at any given TLC threshold; for example, the 12-month risk of mortality at a TLC threshold of 3000 cells/µl was 29% for infants aged 6 months compared to 11% at age 2.5 years. However, the 12-month risk of death did not significantly vary by the TLC threshold. TLC and CD4% were poor predictors of one year mortality for children under 12 months. CD4% and HIV-1 RNA were both significantly associated with the risk of death at a 95% confidence level. For example, decrease in CD4% of 10% was associated with a 1.68 fold increase in risk of death and a one-log increase in HIV-1 RNA was associated with a 2.2 fold increase in risk of death. However, decreases of 1000 cells/µl in TLC were associated with only a 1.1 fold increase in risk of death, and this HR was not significant.

Paper III and IV

ILA cohort
A cohort of HIV-1 infected children aged 6 months to 13 years initiated on HAART using the 2002 WHO ART guidelines for resource-limited settings. Clinical and laboratory data from a total of 124 and 92 children were analyzed in paper III and IV, respectively.

Paper III
A total of 124 HIV-1 infected children with a median age of 5.0 years (IQR 2.1-7.0) and 61/124(49%) female were enrolled and initiated on HAART. The children were more likely to be stunted than underweight, with a median HAZ of -2.0 (IQR -2.9, -1.2) and WAZ of -1.2 (IQR -2.1, -0.5) at baseline. The median CD4% and $\log_{10}$ HIV-1 RNA were 11.75% (IQR 7.5–18.0) and 5.55 (5.2–5.8) copies/ml, respectively. On the basis of treatment outcome, the children fell into the following groups:

- 80 (65%) virological and immunological success – VS/IS
- 27 (22%) virological success and immunological failure –VS/IF
- 10 (8%) virological failure and immunological success – VF/IS
- 7 (5%) virological and immunological failure – VF/IF

Of the children, 107/124(86%) had an undetectable viral load (< 400 copies/ml) and 90/124(73%) had complete immune restoration after 48 weeks of HAART. By the end of the study, all the treatment outcome groups had a significant increase in mean growth z scores regardless of their virological and immunological treatment outcome. The overall mean WAZ and HAZ scores increased by 48 weeks from -1.14 (SD) and -2.06 (SD) at baseline to + 0.6 (SD) and -0.41(SD), respectively (p=0.001). The VS/IS and VF/IS groups both had significant improvements in mean HAZ and WAZ scores on therapy, suggesting that complete viral suppression is not a requirement for an initial increase in weight and height (Fig. 1. Paper III). When the baseline characteristics were placed into a univariate model for association with the successful

treatment group (VS/IS) compared to the other treatment outcome groups, only CD4% at baseline was significantly associated with a successful treatment outcome. In the multivariate model, age and WHO clinical stage were also associated with a successful treatment outcome after adjusting for sex, HIV-1 RNA, CD4%, WAZ and HAZ.

Paper IV

A total of 92 children fulfilled the study inclusion criteria and were enrolled into the study, with 44 in the sdNVP exposed group and 48 in the non-exposed cohort. Baseline demographic data was available for all 92 children, but only 90 children had baseline HIV-1 RNAs. The median duration of follow-up for all children was 72 weeks (range 48–96 weeks). However, we only included time-points up to 48 weeks for this analysis where both cohorts had similar and complete data. Ninety-five percent (85/92) of all study children completed the 48 weeks of follow-up on HAART.

The NVP exposed cohort was significantly younger than the NVP unexposed cohort (median 1.7 vs. 7.8 years, p = 0.38 [Table 2. Paper IV]. At baseline, the NVP exposed group had more advanced HIV disease (WHO stage III 54% vs. 2%) than the non-NVP exposed cohort based on both virological and clinical assessment [Table 2. Paper IV]. However, as expected, the NVP exposed group which was younger, had a higher baseline CD4% (14% vs. 8.5%) compared to the relatively older NVP non-exposed group.

At week 12, 24 and 48, the proportion of HIV-1 infected children with <400 copies/ml (virological treatment success) in those children exposed to sdNVP was 26/43(61%), 34/42(81%) and 33/44(75%), respectively. In those children not exposed to sdNVP, virological success occurred in 29/45(64%), 34/45(76%) and 35/44(80%), at the same time-points [Log-Rank Test p-value =0.8 through 48 weeks (Figure 2. Paper IV)]. By 48 weeks after HAART initiation, over 75% of both cohorts had a viral load <400 copies/ml. In multivariate analysis, none of the baseline factors including sdNVP exposure had an effect on the outcome of virological treatment success (Table 3. Paper IV).

Compared to the baseline, there was a significant and brisk increase in CD4% in both cohorts. The average trend of CD4% and absolute CD4 cell count response over 48 weeks for the 2 cohorts are presented in (Figures 4a and 4b in Paper IV). In the NVP exposed cohort, the mean baseline CD4% was 14%, and there was a brisk and robust mean response in CD4% of 20 percentage points at week 48 on HAART. In the non-NVP exposed cohort, the mean baseline CD4 percent was 8%, with a mean CD4% increase of 19% percentage points at 48 weeks.

Discussion

The overall aim of the studies in this thesis was to describe the natural history of paediatric HIV-1 infection, identify potential laboratory surrogate markers to predict death and document the early response to antiretroviral therapy in HIV-1 infected Ugandan children. All 4 papers used a prospective cohort design with 5 years (Papers I and II) and one year (Papers III and IV) of study follow-up.

For Paper I and II, the parent study (HIVNET012) was a randomized clinical trial to prevent mother-to-child HIV transmission in HIV-1 infected Ugandan women. The HIV-1 infected children from this trial had longitudinal laboratory and clinical data collected over the 5 years. For Paper III and IV, the parent study was an observational cohort of HIV-1 infected children who were eligible for antiretroviral therapy. These infected children were followed for a minimum period of 48 weeks after initiation of ART and clinical and laboratory measurements including height, weight, CD4%/count and viral load were documented.

This chapter will discuss the methodological issues of the different papers, the implications of the studies and overall conclusions drawn from the thesis.

Study methodological considerations

Paper I and II

A prospective birth cohort is an appropriate study design to document the natural history of perinatally HIV-1 infected children and to assess the utility of laboratory surrogate markers for predicting risk of death in children from resource-limited settings. Cohort studies tend to have stronger evidence of causality and are less subject to biases due to selection, recall and measurement errors. The study design and analyses were appropriate for the objectives of the study, to determine the strength and degree of association of the TLC and CD4% measurements, and to check if a certain TLC cut-off could predict risk of death in the subsequent 12 months.

The main limitation of the study was the high mortality in the first year of life leading to fewer data-points as the children became older. Survival bias may have occurred in this cohort of infected children because those children who survived beyond the first year of life were less likely to die and may have had higher TLC. In addition, the CD4 % – the gold standard – does not predict death in children under one year of age, where there is a larger sample size and greater power to detect a difference. Most of the children had high TLC as has been reported in other African children, and so we were unable to correlate TLC and CD4% at the lower levels of TLC where correlation might have been higher.

Bias
All the children who became HIV-1 infected during the clinical trial were followed and had data available for analysis reducing the potential selection bias. However, those who survived had more data-points compared to those who died in the first year of life. In addition, the follow-up rates were very good with <5% loss to follow-up after 5 years so that there was no bias due to those in follow-up being different from those lost to follow-up. Measurement errors could results from laboratory measurements of HIV-1 RNA, TLC and CD4% and counts. To reduce the measurement bias, for example the diagnosis of HIV-1 infection in children was done using HIV-1 RNA PCR and a repeat test was done for those who were found to be PCR-positive. All the HIV infected children also had HIV-1 RNA measurements every 6 months to monitor viral load over time. All the laboratory tests were standardized using quality assurance and controls as required for a CAP certified laboratory.

Validity
The internal validity of the study was good because of the nature of the design and low loss to follow-up. The study also had external validity and could be generalized to other African HIV-1 infected children birth cohorts although our mortality rate may have been slightly lower because of the care provided for the children during the clinical trial. In addition, our results were consistent with cohorts of other African children enrolled in perinatal HIV prevention trials.

Paper III and Paper IV

The study design for both these papers was a prospective observational cohort of HIV-1 infected children aged 6 months to 13 years, initiated on antiretroviral therapy and followed for a minimum of 48 weeks. The strength of this design was the longitudinal nature of the clinical and laboratory data collected over time with good follow-up rates ensuring that all children, except those who died had a similar follow-up period. The follow-up rates were high (98% Paper III and 95% Paper IV) in both groups, and this reduced the potential selection bias which could occur if there was a high loss to follow-up and unknown treatment outcome of a subset of the cohort. Despite being an observational cohort the choice of the longitudinal measurement of viral load and CD4%/counts as surrogate markers for good treatment outcome and is a reliable, standardized and previously reported method for documenting treatment outcome in HIV-1 infected adults and children. Therefore the results of this non-randomized trial could be compared to other treatment trials, with the caveat that there were significant baseline differences that could have affected treatment outcome.

In this study (Paper IV), however, the sdNVP exposed younger children would have been expected to have a higher virological failure rate when compared to the older non-NVP exposed children, but the 2 groups had similar rates of virological suppression after 48 weeks of HAART. Another limitation was the short duration of follow-up (only 48 weeks for all children), we were not able to determine if the success in treatment outcome was sustained beyond the first year of therapy.

This observational cohort design had the potential for selection bias including differences in baseline characteristics which may affect treatment outcome. However, we attempted to control for confounders such as age, baseline viral load and CD4 cell count in the analysis by using logistic regression. A randomized controlled trial would have been a better study design with all children who were exposed to sdNVP being randomized to receive a NVP based HAART regimen or a non NVP based regimen. With a randomized study design the selection bias would be eliminated and the main difference between the 2 arms would be the treatment regimen received. However, we were unable to randomize children to exposure and non-exposure to sdNVP at birth, so this impacted the outcome of the results. The NVP exposed children were younger because the PMTCT program began in the year 2000, so all exposed children were below 4 years, and the non-exposed were older long term survivors.

To reduce the confounding by age, that impacts both viral load and CD4%, we could have matched the sdNVP exposed children to the non-NVP exposed by age. Therefore we compared 2 different populations, which had baseline differences that could have affected the treatment outcomes. However, one would have expected the younger NVP exposed cohort, with a higher viral load and potentially higher levels of resistant mutations, to have had a higher virological failure rate. In our study the 2 groups (NVP exposed and non-exposed) were comparable in virological treatment outcome, and we attempted to control for confounders in the analysis by using logistic regression. The best study design would have been a randomized controlled study (P1060) where the baseline characteristics would be similar and controlled by randomization. This study was subsequently conducted at MU-JHU as part of a multicentre trial of the IMPAACT network, National Institutes of Health, USA.

Implications of thesis findings

Infant and child survival

Infant survival in resource-limited settings is highly dependent on the survival and health of the mother, because the mother not only provides adequate early nutrition through breastfeeding but also improves access to care and treatment [84]. In our cohort of HIV-1 infected children (Paper I), the maternal viral load at delivery was associated with infant mortality, consistent with previous reports [39]. A higher viral load is found in women who are sicker and may not be able to provide adequate nutrition through their breast milk and/or adequate care and treatment for their infants. Maternal mortality was not associated with infant mortality in our study, but this is probably due to the small sample size and the few maternal deaths during the first 18 months of follow-up. Unlike adults with HIV-1 infection, infected infants may die regardless of the degree of immune suppression, with no specific risk factors identifying those at highest risk of dying [85]. Without access to ART, the provision of routine care and treatment for acute illnesses, immunization and nutritional support do not significantly improve the overall survival of HIV-1 infected children. The frequent and severe infectious diseases, poor response to treatment of common childhood

illnesses, underlying severe malnutrition and limited access to health care leads to a high mortality rate in HIV-1 infected children [5].

Most studies have reported a higher mortality rate in children infected *in utero* compared to those infected postnatally through breastfeeding [30, 86] The recent pooled analysis and mathematical modelling by Marston *et al* of the net infant and child survival from multiple perinatal HIV prevention trials in Africa showed a much higher mortality rate for infants infected perinatally compared to those infected postnatally through breastfeeding [86]. Our study (Paper I) showed a slightly higher mortality rate in children infected *in utero* compared to those infected later, but this difference did not reach statistical significance. The findings from our study were similar to the pooled analysis with the *in utero* infected children having a higher mortality rate than those infected postnatally, but because of the small numbers, the study lacked the power to detect a significant difference between the 2 groups. In addition, the classification of the perinatal/*in utero* vs. postnatal infection groups in our study was different from the definition in the pooled analysis. Those infants who were infected *in utero,* but had a negative HIV test at birth, were classified as postnatally infected in our study and this may have affected the overall mortality in the postnatal group. In the pooled analysis, the perinatally infected group included those infants infected *in utero* (HIV-1 positive a birth) as well as those infants who became infected during the first 4 weeks of life. The infants in our study had a much lower mortality with one year survival of 0.68 and 0.82 in the *in utero* and postnatal groups compared to 0.49 and 0.76 in the pooled analysis groups, respectively [86]. The lower mortality rate in our study cohort may be related to the specific clinical care and treatment provided to the infants in our study and the regional differences in infant mortality.

Regardless of the timing of HIV-1 infection, the infected African infant has an unacceptably high mortality. The CHER trial conducted in South Africa demonstrated a significant reduction in mortality when HIV-1 infected infants were initiated on ART within the first few months of life [87]. From this data and other studies, WHO now recommends that all infected children below 2 years of age should be initiated on ART regardless of CD4 cell count or HIV clinical stage [6].

Surrogate markers of disease progression and death

The CD4 cell count is a predictor of death in infected adults and older children but access to CD4 cell counts is not always readily available in many resource-limited settings [88-90]. Simple laboratory tests including haemoglobin, albumin, p24 Ag and total lymphocyte counts were considered potential surrogate markers for disease progression and death for resource-limited settings [85]. However, in resource-limited settings several studies including our own Paper II have found that TLC did not correlate with CD4 cell count and could not be used as a surrogate marker for CD4%/count or to determine those infected children at highest risk of death [91, 92]. Because of the high background mortality rate in children under 2 years, many infected children die before there is evidence of severe immune suppression, decreased TLC or advanced HIV-1 disease. In addition, African children tend to have a lower

white cell count, but have a higher total lymphocyte count, leading to poor correlation of TLC and CD4 cell count [93, 94]. In view of the accumulated data about the poor correlation of TLC and CD4% in resource-limited settings, the WHO ART guidelines were revised to exclude the TLC. In addition, they recommend that all HIV-1 infected children below 2 years of age should be initiated on ART regardless of CD4% or clinical staging, and the CD4 cell cut-offs for initiating ART in older children were lowered [6]. These changes in the guidelines of when to initiate ART in children should lead to children initiating ART prior to severe immuno-suppression; which would improve overall ARV treatment outcomes [57].

Table 6. WHO Antiretroviral therapy guidelines [6] for initiation of ART in children 2010[6].

Immune marker	Age-specific recommendation to initiate ART		
	<24m	**24 - 59m**	**>5 yrs**
CD4 percent	All	<25%	
CD4 count/mm^3	All	<750 cells	<350 cells

Clinical criteria for ART = WHO clinical stage 3 and 4

These findings regarding the utility of the TLC in resource-limited settings suggest that some strategies for monitoring HIV-1 infected children in resource-rich countries cannot be directly transferred to resource-limited settings without prior validation. *Therefore different strategies are needed to identify children at highest risk of death and to monitor disease progression and response to ART in resource-limited settings.*

Antiretroviral therapy and growth

Antiretroviral therapy improves the survival, growth and overall quality of life of HIV infected children [3, 95-98]. The delay in initiating ART is associated with poorer immune and growth responses and subsequent higher rates of virological failure [59]. Despite incomplete viral suppression on ART, many children gain weight and their immune recovery is maintained for a period of time [58]. Our study findings (Paper III) were consistent with other reports where complete viral suppression on ART was not a prerequisite for growth and immune response in children [59, 99]. In our study (Paper III), the older children were less likely to have a significant increase in height and the younger children had a more robust growth response on ART, despite incomplete virological suppression. Incomplete viral suppression with adequate growth and immune restoration in children on ART may be acceptable in resource-limited settings where there are limited treatment options including access to more expensive second line ARV regimens [58]. The challenge of persistent viraemia on ART is the emergence of drug resistant virus, which ultimately leads to immunological and clinical failure with further limitations in second line treatment options [100, 101].

HIV drug resistance after exposure to sdNVP at birth

Exposure to sdNVP at birth leads to emergence of NVP resistant mutations in about 50 % of the HIV-1 infected infants [68, 102]. The emergence of resistance mutations after exposure to sd NVP at birth may impact future treatment options with a NVP containing regimen. In our study of 92 children (Paper IV) with a median age of 1.7 years, sdNVP exposed and non-exposed children had a similar virological treatment response to a NVP containing ART regimen. However, in the P1060 study, subsequent treatment with a NVP containing ART regimen in sdNVP exposed infants lead to higher virological failure rates after 24 weeks of therapy compared to those children who received a PI-based regimen [70]. The P1060 study was a prospective cohort study HIV-1 infected ART naive children who were NVP exposed (cohort 1) and non-exposed (cohort II). They were randomized to receive a NVP based regimen or a Lopinavir/ritonavir based ARV regimen. In both cohorts the NVP based regimen had higher virological failure rates (40.1% vs. 18.6% p=0.001) when compared to the PI-based regimen [70]. This difference may be related to the younger age of children enrolled in the P1060 study compared to our study children. In this randomized clinical trial, despite higher virological failure rates in those children on a NVP based regimen there were some children who were exposed to sdNVP and did respond to the NVP based regimen [70]. In our study (Paper IV), older children (median age 7.8 years) on a NVP-containing regimen had similar virological success rates to those children (median age=1.7 years) who were exposed to sdNVP at birth. This is consistent with fading of the resistant virus over time, with the older children having their virus revert back to wild-type virus, which would be sensitive to NVP [68]. In contrast to the randomized controlled trial where the majority of the children were below three years of age, our small observational cohort (Paper IV) had older children who were less likely to still have resistant virus and would therefore be more likely to respond to a NVP containing regimen.

It is not possible to predict which sdNVP exposed child will have virological success on a NVP-based regimen unless one has access to HIV resistance testing, not currently available in most African countries. Therefore WHO ART guidelines for infants and children recommend that all infants exposed to sdNVP should be initiated on a PI-based regimen [6]. LPV/r, the PI available in most resource-poor settings, is expensive and more difficult to administer in younger children, where the syrup formulation had a short shelf-life unless refrigerated and the Alluvia® tablet cannot be crushed (Lopinavir/ritonavir package insert). Because PIs are not readily accessible in some African countries, infected children under 2 years of age do not have access to the WHO recommended first line ART regimen. Our study findings and other reports suggest that those children who were exposed to sdNVP should not be denied access to ART, when PIs are not available, because some children may still have a successful treatment outcome on a NVP based regimen.

A strategy which could be used in resource-limited settings to spare the PIs for second line therapy, would be to initiate NVP exposed infants on a PI-based regimen and then

later switch to a NNRTI, when the children have achieved complete viral suppression and potentially the NNRTI resistance mutations would have faded. A recent randomized controlled trial of switching to a NVP based regimen after initiating a PI based regimen in children exposed to sdNVP at birth found that a greater number of children had a viral load < 50 copies/ml after 52 weeks (p=0.02), in the switch group compared to those children who stayed on the PI regimen [103]. However, the switch group had more children with a viral load >1000 copies/ml. Therefore, this treatment strategy may be useful in protecting the PI regimens for future second line therapy in resource-limited settings.

As well as providing care and treatment for HIV-1 infected children, there is an urgent need to prevent more infants from becoming infected. The ultimate solution for paediatric AIDS is to eliminate mother-to-child HIV transmission through the scale-up of PMTCT programs in resource-limited countries, in order to significantly reduce the number of infants newly infected each year. We have the knowledge and skills required to scale-up PMTCT services, but implementation has been limited with <50% of pregnant women accessing PMTCT services worldwide (1). The UN agencies have committed themselves to the virtual elimination of paediatric HIV-1 infection, which is <5% transmission of HIV from mother to infant. Currently, most ART and PMTCT services in African countries are highly dependent on donor support including Global Fund for TB, HIV and malaria, and PEPFAR. It is not clear what strategies individual countries have planned for the sustainability of these programs and services. Universal coverage will only be achieved if resource-constrained countries can make a political, civil society and individual commitment to provide, monitor, integrate and utilize the infrastructure for PMTCT services including improvement of the overall maternal and child health services.

Policy implications

The findings from these studies have significant policy implications and highlight the importance of conducting locally relevant paediatric HIV research that can inform WHO and national HIV/AIDS policy. The natural history and mortality data (Paper I) are very important for developing better understanding of the pathogenesis of paediatric HIV-1 in Africa (Paper I). This study is no longer ethical in the era of ART and yet the data provides information that can be used to develop innovative strategies to improve paediatric HIV care and treatment in resource-limited settings. The very high mortality rate in children and additional studies documenting the benefit of early ART in infants has led the WHO to recommend initiation of ART in all HIV-1 infected children under 2 years of age, regardless of CD4% or WHO clinical stage. The findings of poor correlation between TLC and CD4% (Paper II) were consistent with a large study from Africa, and informed WHO policy to withdraw the TLC from the immune criteria for ART initiation in resource-limited settings. These 2 studies highlight the urgent need to implement strategies to prevent common childhood illnesses by providing access to pneumococcal and rotavirus vaccines. In addition, national scale-up of PMTCT services and early infant HIV diagnosis is essential for identification of infected infants and referral for appropriate care and treatment.

Most children who initiate ART gain weight rapidly even if they fail to completely suppress the virus. Therefore, weight gain alone may not determine treatment success in HIV-1 infected children on ART as most children continue to gain weight and maintain a high CD4%, despite virological failure. In rural settings, however, weight gain may be the only measurement that is feasible for documentation of early treatment response when CD4% and viral load monitoring are not accessible. The WHO currently recommends use of weight as a surrogate for treatment response in centres where CD4% testing is not available [6]. Simple point of care CD4% tests are urgently needed to monitor treatment response in resource-limited settings.

After release of the P1060 results which demonstrated that a PI-based ART regimen in NVP exposed infants was superior to a NVP based regimen, WHO now recommends that all NVP exposed infants should initiate ART with a PI-based ART regimen. It is important to note that about 60% of the children on the NVP based regimen had a successful treatment outcome with an undetectable viral load at 24 weeks after ART initiation. In addition, our data (Paper IV) demonstrated similar ARV treatment response in older children exposed and non-exposed to sdNVP at birth. Therefore, in resource-limited settings the use of a NVP based regimen in NVP exposed infants may be considered when PI regimens are unavailable.

The ultimate solution to paediatric HIV-1 infection in resource-limited settings is for countries to make a commitment to scale-up PMTCT services in order to reduce the number of newly infected infants, identify infected infants early and refer them for initiation of ART.

Future research

Developing simple surrogate markers to determine risk of disease progression and death in resource-limited settings are still needed. A simple point of care (POC) test to predict children with the highest risk of dying is urgently needed where the CD4 cell counts are not available. When older children who are stunted are not initiated on ART until eligible, they tend to remain stunted and do not achieve physical maturity despite treatment. Therefore research to determine if the earlier initiation of ART in older children who are stunted, but not yet eligible for ART, could improve growth and maturity remains a priority. Data on the long term outcomes of ART in children from resource-limited settings, including growth, cognitive development and puberty is still needed. In addition, documenting long term complications of ART in resource-limited settings is still needed including the development of strategies to prevent or reduce the complications of ARV drugs.

Developing simplified regimens, additional paediatric ARV fixed-dose combinations and simple strategies for ART delivery and the monitoring of children remains a priority for the future sustainability of ART programs [104]. Simple POC tests to measure viral load could include above or below a certain threshold so as to develop an appropriate cut-off level as opposed to specific HIV-1 RNA copies/ml. A cut-off of <5,000 copies/ml or <10,000 copies/ml and >100,000 copies/ml would be ideal to

monitor those on ART. Strategies to improve adherence in older children are still needed in the community including the setting up of adherence-support peer groups and age appropriate motivational rewards for good adherence. Operational research to improve delivery of paediatric services and retention in care and treatment should be considered including innovative strategies for service delivery. In the future, innovations such as ARVs administered orally or parentally once a month would be useful for older children who struggle with adherence. A life-time of treatment is difficult in a high-income country, but almost impossible in resource-constrained settings, unless different strategies are employed. Development of simplified ART regimens and delivery services, utilization of family and community support for ART including PLA, implementation of innovative strategies for adherence, is critical for the scale-up of ART in Uganda, in an already overloaded health care system.

Conclusions

1. HIV-1 infected Ugandan children had a very high mortality rate during the first two years of life regardless of the time of HIV-1 acquisition, and no specific maternal or infant factors could predict death except the maternal viral load at delivery.

2. The total lymphocyte count is not a surrogate marker for the risk of death in the first 12 months for HIV-1 infected Ugandan children, and is not useful in determining which children are at risk of death and in urgent need of ART.

3. Early initiation of ART, at a young age and prior to severe immuno-suppression, is critical for ensuring adequate growth and an immune response in HIV-1 infected children. However, complete viral suppression is not a prerequisite for adequate early growth and immune responses on ART.

4. Older infants and children had an adequate response to a NVP containing ARV regimen despite exposure to sdNVP at birth, suggesting that NVP based ART may still be an option for older children, even if they were exposed to nevirapine at birth.

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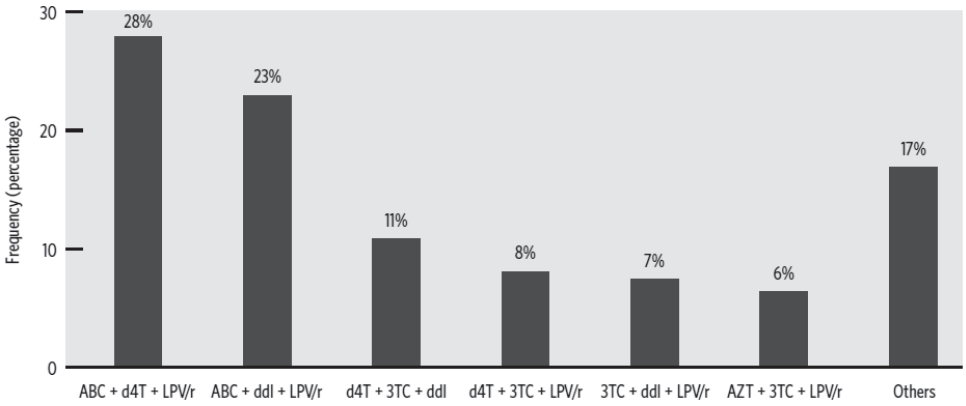
ERRATA LIST

On page 21 Figure 2 was:



Should read (and have been replaced with):