PAEDIATRIC HIV INFECTION IN MAPUTO, MOZAMBIQUE
HORIZONTAL TRANSMISSION, TREATMENT OUTCOMES AND DRUG RESISTANCE

PAULA VAZ

Stockholm 2010
In the memory of my late mother, Rita
ABSTRACT

This thesis describes observational studies performed at the main reference centre for paediatric HIV care and management in Mozambique, which is one of the countries most strongly affected by the AIDS pandemic. These studies focused on horizontal transmission, treatment outcomes and paediatric antiretroviral drug resistance in a cohort of HIV-1-infected children.

The majority of children infected by HIV get the infection from their mothers during pregnancy, delivery or through breastfeeding, in what is known as mother-to-child transmission of HIV-1. However, some of them, in a percentage that is not well ascertained in resource-constrained settings, can be infected through other modes. In the first paper, we explored different risk factors that might have contributed to HIV infection in a small cohort of HIV-infected children born to HIV non-infected mothers. A case control study strongly suggested health care as the possible source of infection for many of these children, with blood transfusion being significantly associated with HIV infection.

Paediatric antiretroviral treatment (ART) has been available for children in Mozambique since December 2003. During the period between December 2003 and December 2008, more than 4700 children aged 0-18 years were recruited, and 1125 received ART in our centre. Survival frequency was 87% after 60 months of antiretroviral treatment. The median follow-up time after ART initiation in the cohort was 24.4 months (IQR 13.5-35.7). After 5 years of ART, 710 children (98.1%) were on first-line treatment, and 21 children (1.9%) were on second-line treatment. After 12 months on ART, there was an improvement in mean (SD) CD4% from 12.3 (6.3) to 27.7 (8.8) (p<0.001). A viral load (VL) cross-sectional evaluation was made in 2006 in 495 children on ART; 72.7% had a VL of < 50 copies/mL. These clinical outcomes are encouraging and provide stronger evidence in support of wider scale-up of long-term treatment of HIV-infected children with NNRTI-based first-line antiretroviral drugs in sub-Saharan Africa.

Resistance to paediatric antiretroviral drugs in children in sub-Saharan Africa has not been documented. We performed genotypic resistance tests in 84 available samples from 135 treated children with VL ≥50 copies/mL among children in whom cross-sectional viral load assessment was carried out in 2006. Approximately 92% of the viruses found in this cohort were resistant to lamivudine and/or nevirapine, and 15% were resistant to stavudine. Twenty children (24%) harboured virus with an extended spectrum of cross-resistance. A multivariate analysis revealed that the only factor associated with this extended resistance profile was treatment duration (OR: 6.67 [95% CI 1.24; 35.93], p<0.05 for treatment >24 months), with a per month increase of 1.09 [1.02-1.16] (p=0.0065).

Opportunistic infections are frequent in HIV-1-infected children, as are oncology-related infections. To date, there are no published reports investigating clinical outcomes of HIV-1-infected children with Kaposis’s sarcoma. We described chemotherapy and ART outcomes in a cohort of 28 children with histological confirmed AIDS-associated Kaposis’s sarcoma (AKS). The cohort included twenty (71%) males and 8 females who were moderately ill, severely immunosuppressed children. The mean patient age was 8.3 (range 2-16) years, and the mean (SD) follow-up time was 27.3 (14.9) months. All patients were ART naive. Twenty-four children on ART were alive at 6 months after completing chemotherapy, corresponding to a survival rate of 85.7%. The CD4 % increased from a mean (SD) of 16% (9.2) to 29.2% (27.1) before initiation and after cessation of chemotherapy (p=0.02). Our findings in children confirmed what has been observed in adults: a good clinical outcome with an acceptable toxicity of combined highly active antiretroviral therapy (HAART) and chemotherapy in ART-naive, HIV-1-infected children with associated Kaposis’s sarcoma.


Related publications:


### LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIDS</td>
<td>Acquired immunodeficiency syndrome</td>
</tr>
<tr>
<td>AKS</td>
<td>AIDS-associated Kaposi’s sarcoma</td>
</tr>
<tr>
<td>ART</td>
<td>Antiretroviral therapy</td>
</tr>
<tr>
<td>CCR5</td>
<td>Chemokine receptor 5</td>
</tr>
<tr>
<td>CDC</td>
<td>Centers for Disease Control</td>
</tr>
<tr>
<td>CTL</td>
<td>Cytotoxic T lymphocytes</td>
</tr>
<tr>
<td>CXCR4</td>
<td>CXC chemokine receptor</td>
</tr>
<tr>
<td>DC</td>
<td>Dendritic cells</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>DREAM</td>
<td>Drug resource enhancement against AIDS and malnutrition</td>
</tr>
<tr>
<td>ECS</td>
<td>European Collaborative study</td>
</tr>
<tr>
<td>EKS</td>
<td>Endemic Kaposi’s sarcoma</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
</tr>
<tr>
<td>env</td>
<td>Envelope gene</td>
</tr>
<tr>
<td>GI</td>
<td>Gastrointestinal tract</td>
</tr>
<tr>
<td>gag</td>
<td>Group specific antigen</td>
</tr>
<tr>
<td>gp41</td>
<td>Glycoprotein 41</td>
</tr>
<tr>
<td>gp120</td>
<td>Glycoprotein 120</td>
</tr>
<tr>
<td>HAART</td>
<td>Highly active antiretroviral therapy</td>
</tr>
<tr>
<td>HiB</td>
<td>Haemophilus influenza type B</td>
</tr>
<tr>
<td>HIV</td>
<td>Human immunodeficiency virus</td>
</tr>
<tr>
<td>HHV8</td>
<td>Human herpes virus type 8</td>
</tr>
<tr>
<td>HLA</td>
<td>Human leukocyte antigen</td>
</tr>
<tr>
<td>IgA</td>
<td>Immunoglobulin type A</td>
</tr>
<tr>
<td>IgG</td>
<td>Immunoglobulin type G</td>
</tr>
<tr>
<td>IgM</td>
<td>Immunoglobulin type M</td>
</tr>
<tr>
<td>IRIS</td>
<td>Immune reconstitution inflammatory syndrome</td>
</tr>
<tr>
<td>KIR</td>
<td>Killer immunoglobulin-like receptors</td>
</tr>
<tr>
<td>KS</td>
<td>Kaposi’s Sarcoma</td>
</tr>
</tbody>
</table>
KSHV  Kaposi Sarcoma associated herpes virus
LIP  Lymphocytic interstitial pneumonitis
MHC  Major histocompatibility complex
MISAU  Ministry of Health in Mozambique
MTCT  Mother-to-child transmission of HIV
nef  Negative factor
NGO  Non-Governmental Organization
NIH  National Institutes for Health
NK  Natural killer cells
NRTI  Nucleoside analogue reverse transcriptase inhibitor
NNRTI  Non-Nucleoside analogue reverse transcriptase inhibitor
OI  Opportunistic infection
PCP  *Pneumocystis jiroveci (former carinii)* Pneumonia
PCR  Polymerase chain reaction
PDH  Paediatric Day Hospital
PI  Protease inhibitor
PACTG  Pediatric AIDS Clinical Trials Group
PENTA  Paediatric European Network for the treatment of AIDS
PMTCT  Prevention of mother-to-child transmission
pol  Polymerase gene
rev  Regulator of RNA transport
RNA  Ribonucleic acid
RT  Reverse transcriptase enzyme
SDF-1  Stromal cell-derived factor 1
SNP  Single nucleotide polymorphisms
STDs  Sexual transmitted diseases
SWAp  Sector-wide approach
tat  Transactivator of viral transcription
TAM  Thymidine analog resistance mutation
TB  Tuberculosis
TCR  T-cell receptor
TLC  Total lymphocyte count
TLR  Toll-like receptor
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>UNAIDS</td>
<td>United Nations Organization for AIDS control</td>
</tr>
<tr>
<td>UNICEF</td>
<td>United Nations Children’s Fund</td>
</tr>
<tr>
<td>vif</td>
<td>Viral infectivity factor</td>
</tr>
<tr>
<td>vpr</td>
<td>Viral protein R</td>
</tr>
<tr>
<td>vpu</td>
<td>Viral protein U</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
</tbody>
</table>
place together with a memorandum of understanding to create a common pool of funds within the treasury. The MISAU and its partners jointly developed a health sector strategic plan and held monthly joint operational meetings of the SWAp, chaired by the MISAU, as well as biannual high-level policy meetings, annual joint evaluations and sector-wide planning and budgeting. Non-governmental organizations (NGOs) are also playing a critical role in service provision, with roll-outs of HIV/AIDS services being implemented in partnership with non-governmental providers [17].

Today, a new mechanism is in place, where the MISAU can contract NGOs directly, improving collaboration, oversight and local ownership. The NGO umbrella organizations are increasingly active in SWAp as recognized partners [17].

In 2002, a national strategy regarding ART was designed, and the government together with the Global Fund, the World Bank-MAP, the Clinton Foundation and other partners made ART available within the national health system, totally free of charge.

Successful implementation of a national treatment plan depends on strong leadership by the Ministry of Health. In the case of Mozambique, consistent, coordinated support for the MISAU HIV/AIDS strategic plan has ensured that the MISAU remains firmly in control. All decisions regarding HIV/AIDS planning, budgeting and service delivery issues are taken up by the SWAp and are thereby integrated into the broader health system [17].

1.1.3.2 Paediatric scaling-up of antiretroviral treatment in Mozambique

Sensitivity and awareness of children’s health needs is frequently lower than for the adult population. As a result, health problems regarding children are often unaddressed or underserved. Consequently, children are underrepresented among ART recipients in many settings worldwide where treatment programs have been established, including Mozambique. Implementation of paediatric ART in resource-limited settings represents specific challenges. These challenges range from inappropriate sensitivity to children’s health issues, a huge and still increasing population of HIV-1 infected children in need of care, economies that are unable to bear the cost of HIV burden and health personnel and institutions that are insufficient and unprepared to face the problems of cultural perception and coping of chronic disease. Nevertheless, Mozambique’s government has made a great effort to focus on
HIV-1-infected children with the implementation of the Paediatric Treatment Strategy. Various partners such as UNICEF, Columbia University (CU-ICAP), Médecins sans Frontières (MSF), Clinton Foundation, Elisabeth Glaser Paediatric Foundation (EGPAF), Health Alliance International (HAI) and others have joined and supported the government in implementing the strategy. It has included the identification and nomination of a leader for paediatric ART implementation as well as the identification and precocious involvement of stakeholders, advocacy and education about paediatric HIV/ART, integration of an ART program in the national health system, establishment of simple, accessible paediatric guidelines for all health professionals, training of all health professionals (nurses, doctors, pharmacists, psychologists), efficient access and referral systems and continuous monitoring and supervision. It has resulted in a huge increase in the number of health facilities providing paediatric ART, from 3 in 2003 to 181 in 2008 (73% of them in rural areas), together with a huge increase in the number of children receiving ART, from less than 50 in 2003 to more than 10,000 in 2009.

1.2 Virology of HIV

The acquired immunodeficiency syndrome known as AIDS was first described in 1981 by Gottlieb [18]. The human immunodeficiency virus (HIV) was identified as the causative agent in 1983 by Francoise Barré-Sinoussi and Luc Montaigner [19], later confirmed by Robert Gallo.

HIV is a retrovirus that is grouped into two types, HIV-1 and HIV-2. HIV-1 occurs worldwide, whereas HIV-2 is mainly observed in West Africa and India. HIV-1 is branched into four groups: Main (M), Outlier (O), Non-M-non-O (N) and the recently discovered P. HIV-1 group M represents the main strains that are circulating worldwide and is further divided into 14 different subtypes, also referred to as clades (A-D, F-H, J-K) and 16 established circulating recombinant forms, which have a different geographical distribution [20-23]. Recombinant virus are the result of superinfection with two or more virus strains [24]. If an inter-subtype recombinant virus is transmitted and becomes one of the circulating strains in the epidemics, it can then be classified as a circulating recombinant form (CRF).

In North America and Europe, subtype B is more common, whereas subtype C is the most widely spread strain worldwide [21, 22]. Subtype C accounts for 56% of all HIV-1 infections worldwide [25, 26]. Although both subtypes emerged at the same time,
around the early 1960s, they show different growth patterns. The subtype C epidemic in sub-Saharan Africa is doubling every 2.4 years at a constant rate that is almost half the rate observed in the early stages of the subtype B epidemic in Western Europe and the US [27]. The subtype B epidemic in Western Europe and the US in the 1980s spread twice as fast as the current subtype C epidemic in the sub-Saharan region, and after a rapid expansion over 15 years, its growth rate has declined [27]. It has been suggested that the differences in growth rates of the two subtypes are not due to infectivity but rather to differences in transmission networks, in particular, the distribution of time between transmissions [27]. Sub-Saharan Africa is particularly vulnerable to a heterosexually transmitted HIV epidemic due to the variation in partner changes, low reported condom use [28] and high prevalence of sexually transmitted diseases, particularly herpes simplex 2 [29].

In Africa, there are various HIV-1 subtypes, including recombinant viruses [21, 22, 24, 30, 31]. The main circulating strain in Mozambique is subtype C [30-34], although other subtypes have been identified less frequently, subtype A in 11% of cases [32], subtype D in 7.1% of cases [32], subtype G in 3.8% of cases [30] and, to a much lesser extent, some mosaic and recombinant forms [30-32].

The HIV-1 virus can also be classified according to the use of co-receptors. Viruses that preferentially use the CCR5 receptor are called R5 viruses, and those using the CXCR4 receptor are called X4 viruses. Viruses using both co-receptors are dual-tropic R5/X4 viruses [35]. R5 viruses, also known as macrophage-tropic and non-syncytium-inducing, predominate during early infection, whereas X4 viruses, also known as T cell-tropic and syncytium-inducing viruses, are more frequently found in late stages of the disease [36]. In Mozambique, a study has shown that env sequences are predominantly CCR5, although CXCR4 were also identified in 13% of patients [33].

1.2.1 HIV structure
HIV-1 belongs to the Retroviridae family and is found in the Lentivirus genus. The virus is spherical, with an approximate diameter of 100 nm. It has an outer lipid bilayer called the envelope that originates from the host cell and is formed during budding. The lipid bilayer surrounds an inner nucleocapsid, which in turn surrounds the viral nucleic acid. The envelope protein (gp160) is glycosylated and processed in the endoplasmic reticulum and Golgi complex, where it is cleaved into the surface
proteins glycoprotein 41 (gp41) and glycoprotein 120 (gp120). Gp120 binds to the CD4 receptors present on the target cell (T cells, macrophages, dendritic and microglia cells).

The virion contains two copies of a single-stranded ribonucleic acid (RNA). The genome contains three major genes: group specific antigen (**gag**), polymerase (**pol**) and envelope (**env**). In addition, HIV carries the following regulatory genes: transactivator of viral transcription (**tat**), regulator of RNA transport (**rev**). It also carries the accessory genes viral infectivity factor (**vif**), viral protein R (**vpr**), negative factor (**nef**) and viral protein U (**vpu**).

### 1.2.2 HIV replication

The major targets for HIV-1 are cells expressing the CD4 surface antigen, which are predominantly T-helper cells (lymphocytes) and cells of the monocyte/macrophage lineage. As mentioned above, co-receptors are also important for virus entry into the host cell, as the absence of CCR5 has been shown to be a protective factor against HIV-1 infection [37, 38]. HIV-1 enters the host cell in a multi-step process mediated by the **env** protein. Infection begins with gp120 binding to the CD4 molecule on the target cell, which then undergoes conformational changes that enable the protein to bind to CCR5, CXCR4 or other co-receptors. Subsequently, a fusion of viral and cellular membranes occurs, mediated by gp41, which enables the viral capsid and genome to enter the host cell. Upon entering the cell, the protease enzyme produces the reverse transcriptase and ribonuclease enzymes responsible for synthesising the single-stranded DNA molecules and primers necessary to produce the complementary DNA strand. The reverse transcriptase enzyme (RT) transcribes viral RNA to DNA. After reverse transcription, the retroviral genome will be integrated into the host cell DNA in a process facilitated by the viral integrase. The integrated DNA, called a provirus, can remain in a latent stage or be activated and form new infectious particles. The provirus is used as a template for genomic RNA and messenger RNA production, which are translated into viral proteins. The new viral RNA forms the genetic material for the next generation of virus. The final step of replication is assembly of new virions at the plasma membrane that bud out from the host cell. At this stage, the virus has completed its maturation and is ready to infect other cells. Because reverse transcriptase lacks proofreading ability, it results in considerable base-to-base
variability. The high mutation rate combined with a high reproductive rate results in substantial evolution and subsequent treatment resistance.

Studies performed in children to assess the dynamics of HIV-1 replication in vertically infected infants have confirmed the central role of HIV-1 replication in the pathogenesis of vertical HIV-1 infection [39]. In contrast to what happen in adults following primary infection, where viral levels peak and recline, plasma HIV-1 RNA levels in infants rise and remain high (mean of $10^5$ copies/ml plasma) during the first 2 years of life. After this period, a reduction is observed in vertically infected children that continues through 5-6 years of age [40-42] in the absence of treatment. In infants under 5 months of age, the rate of HIV-1 production and turnover is at least as rapid as those reported for adults; however, in older infants and children, there is a more rapid production and turnover of plasmatic HIV-1 compared to adults [39]. These age-related differences in viral kinetics could be explained by age-related differences in the number of cells able to support productive infection, particularly activated CD4 T cells [39] and a less vigorous or delayed development of specific immune responses against HIV-1, especially antibody-dependent cellular cytotoxicity and cytotoxic T-lymphocyte responses [43-45], in young children.

1.3 Immunopathogenesis of HIV

The HIV-1 infection is characterized by continuous viral replication, chronic immune activation and loss of CD4+ T cells, which leave the patient gradually more susceptible to various opportunistic infections.

It has been suggested that even though the virus remains central in the pathogenesis of AIDS by infecting and killing central memory T cells, immune activation plays an important role in disease progression [36, 46]. Systemic immune activation occurs during the entire disease process, and it has recently been suggested that it is caused by microbial translocation across the damaged gastrointestinal (GI)-mucosal barrier [47-50]. Although it has been long known that enteropathy is associated with progressive disease, only very recently could the relationship between the detrimental effects of HIV-1 on the GI tract during both acute and chronic phases of disease be better understood [47]. The majority of CD4+ T cells reside in the GI tract, and as a result of infection, there is a major depletion of these cells, thus representing an important assault to the immune system [46]. Recent observations have shown that
Systemic immune activation is key for disease progression, and it has even been suggested to be a better predictor of disease progression than viral load [51, 52]. Antiretroviral treatment reduces viral load and contributes to less GI immune activation, leading to an increase in peripheral blood CD4+ T cells. However, the reconstitution of CD4+ T cells at GI tract level is poor, especially if treatment is not started early [53].

1.4 Immune Responses to HIV Infection

1.4.1 Innate immunity in HIV infection

Innate immunity is always present and is the first line of defence to protect an individual against infection. Innate immunity is conferred by a diverse array of cellular and sub-cellular components that are present in an individual at birth and are composed of different cellular and non-cellular elements. The cellular elements include body surfaces (skin and mucosa), phagocyte cells, macrophages and microglia cells, which represent effective barriers to environmental agents. Cells of the innate system are responsible for a localized host response; they act immediately, in a period of hours, and are responsible for capturing and transporting invading material to the lymphoid organs and presenting it to the adaptive immune system [54]. Cytokines and chemokines released by the cells involved in the innate immune response, attract more cells to the site of action in order to build up an effective reaction against the invading microorganism. Non-cellular elements are the complement system as well as interferon, pattern recognition molecules (such as Toll-like receptors, which bind to various microorganisms) and serum proteins (such as β-lysin, lysozyme, polyamines and kinins). These elements can either directly affect the pathogenic agent or enhance the host reaction to the invader microorganism.

Mucosal tissues are the largest collection of lymphoid tissues in the body, and the majority of T- and B-lymphocytes are localized in the gastrointestinal mucosa [55]. Given that the majority of HIV transmission occurs via mucosal surfaces, the mucosal innate immune responses play a major role in HIV infection by limiting HIV replication and dissemination [56, 57]. However, innate mucosal immunity can be affected by genital infections and intestinal helminthes through inflammation at mucosal sites, increasing the likelihood of HIV-1 acquisition and transmission [58]. Although HIV-1
can traverse epithelial cells and dendritic cells (DC) [59, 60]. CCR5 CD4+ T cells, DC and macrophages are some of the first cells to become productively infected. DC and T cell clusters then drive the infection, with DC migrating to lymph nodes, facilitating the dissemination of HIV and the destruction of 30-60% of CD4+ memory cells in the body in just a few days, particularly in the mucosa and gut [61, 62].

1.4.2 HIV-1 specific cellular immune responses

Cellular immunity is involved in the initial control of virus replication in primary HIV-1 infection. In this context, HIV-1-specific CD8+ T cells are important for controlling viral replication, and it has been shown that their appearance coincides with the initial decline of viral load in primary infections [63], preceding the appearance of neutralizing antibodies. It has also been demonstrated in HIV-infected adults that the functional profile of HIV-specific CD8+ T cells in progressors is limited compared to non-progressors, who consistently maintain highly functional CD8+ [64]. In the same study, the frequency and proportion of the HIV-specific T-cell response with highest functionality (i.e., the ability to produce several cytokines and to de-granulate) inversely correlated with viral load in progressors. Thus, rather than quantity or phenotype, the quality of the CD8+ T-cell functional response serves as an immune correlate of HIV disease progression. In particular, HIV responses directed to gag are associated with better control of HIV disease. CD8+ T cell activity, however, is dependent on CD4+ T cell helper activity. In the absence of antiretroviral treatment, strong, polyclonal, persistent and vigorous HIV-1-specific CD4+ T cell proliferative responses resulted in the elaboration of interferon-gamma and antiviral beta chemokines, which were associated with control of viremia [65]. HIV-infected patients lose the ability of their CD4+ T cells to proliferate in late stages of disease progression. Strong HIV-1-specific proliferative responses were also detected following treatment of acutely infected persons with potent antiviral therapy. Thus, HIV-1-specific helper cells are likely to be important for immunotherapeutic interventions [65].

The development of cellular immune responses among infants is important, especially as T cell function in the foetus and neonate is immature or not fully developed [66]. HIV-specific cellular immune responses can be detected among more than one-third of infants born to HIV-infected mothers [67]. Some studies performed in HIV-1 exposed but uninfected infants have shown both cytotoxic T lymphocyte responses to
HIV-1 epitopes [68-70], lymphocyte proliferation after stimulation with recombinant HIV-1 proteins and HIV-stimulated IL-2 production [71]. In these exposed, non-infected infants, HIV-1-specific CD4+ T cell activity was more frequently detected than CD8+ T cell activity, as expressed by the production of interferon gamma [72]. Nonspecific immune responses were also observed in these HIV-1-exposed, non-infected infants, with elevations in the populations of activated helper T cells and memory helper T cells [72, 73]. The persistence of HIV-specific responses in these infants has not been well described. However, in one study, HIV-specific T cell responses could only be detected up to the age of 6 months, suggesting that these responses wane without antigenic stimulation [74].

Envelope-specific cytotoxic T-lymphocytes are less common in children who acquire the disease vertically than in children who acquire HIV by means of blood transfusion. Among those with vertically acquired disease, cytotoxic T-lymphocytes are least common in those with rapidly progressing disease. Furthermore, precursors of cytotoxic T-lymphocytes that are specific to HIV-1 do not develop in significant numbers until the child is 1 year old.

1.4.3 HIV-1 specific antibody responses

The humoral response in HIV-1 infection occurs early after initial infection, with the appearance of HIV-1-specific binding and neutralizing antibodies. A number of broadly neutralizing human monoclonal antibodies that react with gp120 or gp41 have been identified [75, 76].

In infants, an early serologic response to infection with HIV-1 is normally obscured by the presence of transplacentally acquired maternal HIV antibodies. In an early study performed to assess serological responses in infants of HIV-1-infected women, it was observed that in the neonatal period, infected infants produced only small amounts of HIV-specific IgG antibodies to a restricted number of antigens. However, the amount of HIV-specific immunoglobulin and the number of recognized HIV-1 antigens increased with age; after 6 months, 85% of infected infants had detectable antibodies to two or more viral proteins [77]. Antibodies directed toward gp160 appeared first and were frequently found at all ages, followed by antibodies against gp120 and gp41, whereas antibodies to the pol and gag proteins appeared later and in only a small percentage of infants [77]. In addition, the demonstration of a positive correlation
between the amount of HIV-1 antibodies produced and the percentage of CD4+ T lymphocytes in peripheral blood [77] confirmed the relationship between antibody production and T helper cell function.

In adults, the pattern of IgG subclass reactivity against HIV antigens changes with disease progression [78]. In children, though all IgG classes cross the human placenta [79], the highest reactivities are in the subclasses IgG1 and IgG3 between the ages of 3.5 and 6.5 months [80]. In children, there is also increased production of IgM, IgG and IgA, which have been associated with disease progression [81]. Neutralizing antibodies have been related to the absence of infection in exposed but uninfected infants [82, 83]. Furthermore, the development of broadly neutralizing antibodies is associated with slowed disease progression in adults, children and infants.

1.4.4 Genetic susceptibility to HIV infection

The genetics of hosts and microbes can determine infection and disease outcomes [84]. There is increasing data supporting the idea that host genetic factors are important determinants of HIV-1 susceptibility in MTCT and disease progression [84]. The immune response against HIV-1 is modulated by different host genetic determinants. These determinants are directly or indirectly implicated in virus recognition and immune response amplification (including molecules involved in signalling pathways and cytokine genes) [85]. Virus recognition is made by chemokine receptors, human leukocyte antigen (HLA), T-cell receptor (TCR), antibodies, killer immunoglobulin-like receptors (KIR), toll-like-receptors (TLR) and immune cell trafficking (chemokines and adhesion molecules) [85].

The virus can evolve and adapt in different ways in dissimilar individuals, depending on their genetic structure and concordance; the greater the immune concordance between the virus donor and recipient, the easier it is for the virus to establish itself in the new host [85].

Amongst the various immunogenetic determinants known to influence HIV/AIDS, the HLA system, which is the major hystocompatibility complex (MHC) of humans, represents the central focal point, as it usually presents foreign peptides to the immune system. Hence, MHC is the major stimulus for shaping viral mutations. There are MHC-associated genes that are known to influence HIV infection. At least two
alleles, HLA-B27 and HLA-B57, have been identified as being protective against HIV-1, whereas HLA-B35 and HLA-B53 act as susceptibility factors [85]. During primary HIV-1 infection, viruses using the CCR5 co-receptor are predominantly transmitted, and any polymorphisms (such as single nucleotide polymorphisms (SNP)) that affect CCR5 expression can modify the rate of disease and MTCT [84]. CCR5 is a critical co-receptor in perinatal transmission [86, 87]. It has been shown that a deletion of 32 bp from the coding region of the CCR5 gene (Δ32) can confer almost complete protection against HIV-1 infection in individuals presenting the homozygous mutant genotype [88], while individuals presenting the heterozygous mutant genotype (CCR5-wt/Δ32) are less likely to be infected by HIV-1 and present a slower rate of disease progression [89, 90]. In a cohort of HIV-1-infected children, the presence of the CCR5-wt/Δ32 genotype was associated with higher CD4 percentage and cognitive scores and lower viral loads compared to children without that genotype [91]. By contrast, a genetic variant in the CCR5 promoter region, 59029-A/A, was found in approximately 25% of HIV-1-infected children and was associated with an approximately 50% increase in disease progression[91]. Stromal cell-derived factor 1 (SDF-1), a natural ligand for CXCR4, is associated with slower disease progression in children [92, 93].

Another minor HIV-1 co-receptor, CX3CR1 (a leukocyte chemotactic and adhesion receptor), was associated with more rapid AIDS progression in patients who were homozygous for CX3CR1-M280 [94]. The expression of CX3CR1 and its ligand fractalkine are increased during HIV-1 infection and are reduced when the patient is undergoing effective HAART [95]. In HIV-1-infected children, genetic variants reducing CX3CR1 expression markedly influence the risk and rate of disease, as these children have an increased risk for central nervous system impairment [91].

1.5 Transmission of HIV-1 Infection

HIV-1 can be transmitted through blood and body fluids, for example, by exposure to unsterile intravenous procedures in drug users, blood transfusions, unsterile health care procedures, sexual intercourse (heterosexual or homosexual) and mother-to-child transmission. Initially, in the epidemics in the US and Europe, the main modes of transmission were homosexual intercourse, drug use and blood transfusion, but with the course of the epidemic and the spread in Africa, heterosexual intercourse became
the predominant mode [1]. HIV-1 transmission can be facilitated by several identified risk factors, in which high viral load [96], multiple sexual partners [97] and sexually transmitted diseases [97, 98] play important roles.

1.5.1 Vertical Transmission

Mother-to-child transmission (MTCT) is responsible for more than 90% of paediatric infections worldwide. Children can be infected during pregnancy, in uterus, during delivery or postnataally through breastfeeding. In industrialized countries, before the prophylactic era, the transmission rate from mother to child ranged from 15-25%, provided mothers did not breastfed [99, 100]. However, in Africa, the mother-to-child transmission rates can be much higher, 13-42%; this rate includes breastfeeding as the norm [100-102].

In the absence of breastfeeding, HIV-1 transmission occurs in uterus in 30-40% of cases; the remaining 60-70% occur intra-partum [103, 104]. Postnatal transmission through breastfeeding has been demonstrated in a number of studies [105, 106] and can vary from 14% to 44% [107, 108]. In a meta-analysis that included data from 9 studies investigating late postnatal HIV-1 transmission in breastfed infants, the overall risk of late postnatal transmission was 8.9 transmissions per 100 child-years of breastfeeding [109]. The risk transmission of HIV is higher in early breastfeeding, with the majority of children acquiring the infection in the first 2 months [110], as reported by the HIV network for prevention trials 012 in Uganda.

1.5.1.1 Risk factors

Several factors influence MTCT of HIV-1, including maternal health status, viral and immune response characteristics, obstetric and placental factors, breastfeeding patterns and breast pathology. Early studies have identified maternal advanced disease, measured by CD4 count or AIDS diagnosis, as a high risk factor for HIV-1 transmission from mother to child [111]. Several studies have demonstrated that maternal HIV-1 viral load is a strong independent risk factor for HIV-1 transmission [112],[113],[114]. Furthermore, the levels of free virus in genital secretions [115] or cervical or vaginal HIV-DNA levels were associated with increased infant infection [116]. In addition to viral load, the phenotype of the mother’s virus influences HIV-1 transmission, as it has been suggested that viruses with high replicative capacity and
cytopathogenicity have a higher risk of transmission [117, 118]. It has also been suggested that subtype C viruses were preferentially transmitted in utero compared to other subtypes [119]. Maternal and infant innate immunity, such as chemokines and secretor leucocytes protease inhibitors in breast milk, genital secretions and saliva, as well as HIV-1 specific immune responses, can decrease MTCT of HIV-1 [82]. Maternal HIV-specific neutralizing antibodies may also play a role in preventing MTCT [82], as mothers who present them are less likely to transmit the virus to their offspring. In HIV-1 exposed but uninfected infants, HIV-specific CD4+ T helper cell responses and cytotoxic T-lymphocyte (CTL) responses were associated with protection against MTCT of HIV-1 during delivery and breastfeeding [67, 82]. Natural killer (NK) cells play a traditional role in host defence against virally infected cells, and a recent study demonstrated that HIV-1 maternal and infant NK cell responses are protective against MTCT of HIV-1 [120]. In the US, there was an early report that a duration of membrane rupture greater than 4 hours was associated with a two-fold increased risk of infant HIV-1 infection [121], which was later confirmed by a study from France where other obstetric factors, such as amniocentesis and amnioscopy, preterm labour, sexually transmitted diseases during pregnancy, haemorrhage and blood in the amniotic fluid were associated with an increased risk of transmission [122]. Chorioamnionitis is also associated with a higher risk of HIV-1 transmission [123]. Regarding the mode of delivery, Caesarean section before labour has a protective effect [124]. Finally, breast pathology, such as mastitis or breast abscesses, increases MTCT, and exclusive breastfeeding is associated with a lower incidence of MTCT compared to mixed breastfeeding [74].

1.5.1.2 Prevention of mother-to-child transmission of HIV

Prevention of mother-to-child HIV transmission (PMTCT) is one of the most important issues in preventing the spread of HIV-1. Since the successful results of the ACTG 076 ANRS 024 study in 1993, where zidovudine was given to mothers from the 14-34th week of gestation during pregnancy, intravenously during labour and delivery and orally to the newborn during the first 6 weeks, resulting in a 68% reduction in the risk of perinatal transmission [125], significant progress has been made worldwide. A number of other antiretroviral regimens and strategies have proven successful in reducing PMTCT [126-131]. In 1996, the introduction of highly active antiretroviral
therapies (HAART), a combination of three drugs in which a non-nucleoside reverse transcriptase inhibitor (NNRTI) or protease inhibitor (PI) is administered together with nucleoside reverse transcriptase inhibitors (NRTI), created a revolution in antiretroviral treatment. HAART have completely altered morbidity and mortality in adults and children and have positively impacted PMTCT programs. One example that shows the effect of HAART on PMTCT is the WITS study, where MTCT-transmission rates decreased to 1.2% in mothers receiving HAART compared to mothers receiving zidovudine alone (10.4%) and mothers receiving dual treatment (3.8%) [132]. However, in resource-constrained settings where antenatal care, health infrastructure and staff resources are insufficient, other regimens needed to be implemented that fulfilled criteria such as cost-benefit, feasibility and efficacy. Consequently, studies have been performed to assess the efficacy of shorter courses of zidovudine to prevent MTCT. In non-breastfeeding women, zidovudine treatment from the 36th week of gestation and orally during labour and delivery resulted in a reduction in transmission of 50% [133]. In breastfeeding women in Côte d’Ivoire and Burkina Faso, a reduction in MTCT of 37% was demonstrated at 3-6 months but not later, suggesting that zidovudine did not influence breastfeeding transmission [126, 127]. However, it was the HIVNET 012 study [128] that truly allowed prevention of MTCT (PMTCT) programs in sub-Saharan Africa. In this study, performed in a population of breastfeeding women, there was a 41% reduction of HIV-1 transmission from mother to child with the use of single-dose nevirapine to both mother and infant. The low cost, easy administration and high efficacy of this regimen made it possible for PMTCT programs across sub-Saharan Africa to attain high coverage and benefit hundreds of thousands of women and children. However, the issue of resistance arose, and several studies demonstrated nevirapine resistance in 25-75% of women 2-8 weeks postpartum [134, 135] and 33-87% of infants exposed to single-dose nevirapine [136]. These results made the use of single-dose nevirapine questionable in settings where first-line HAART includes nevirapine. Furthermore, the PETRA study, conducted in three sub-Saharan countries, showed that a combination of zidovudine and lamivudine administered from the 36th gestational week and the first week after delivery resulted in a 63% reduction in MTCT at 6 weeks [131]. A trial in Thailand with non-breastfeeding women receiving zidovudine from the 28th gestational week and including extended infant prophylaxis for 3 days or 6 weeks led to a MTCT rate of 4.7%
and 6.5%, respectively [137], representing good progress in PMTCT. In a subsequent study in Thailand using zidovudine at the 28th week followed by a single dose of nevirapine during labour or delivery, the MTCT rate was 2% in a non-breastfed population [138]. Other interventions, such as antiseptics during labour [139, 140], prophylactic antibiotics to prevent chorioamnionitis [141, 142] or administration of Vitamin A [143-145] failed to show any benefit in reducing MTCT of HIV-1. While several interventions have proven effective in reducing MTCT, most of these benefits seem to be counteracted by breastfeeding.

Breastfeeding poses the major challenge in preventing postnatal MTCT, as the majority of women in sub-Saharan Africa breastfeed their infants [146]. In sub-Saharan Africa, breastfeeding is critical for infant survival but also accounts for HIV-1 transmission in up to 16% of untreated infants that continue breastfeeding into the second year of life [147]. Acceptable, feasible, affordable, sustainable and safe feeding options other than breast milk are scarce in this region. This has led to a number of studies attempting to reduce MTCT through breast milk. These studies have attempted to decrease MTCT through breast milk by treating the mother, the infant or both or choosing a breastfeeding modality (exclusive, mixed or formula).

Studies comparing the risk of HIV-1 transmission in exclusive breastfeeding and mixed feeding were conducted in South Africa, Zimbabwe and Zambia and have demonstrated a lower risk of HIV-1 transmission in exclusive breastfeeding compared to mixed feeding [74, 148-150]. A trial performed in Kenya has shown a significantly lower HIV-1 infection rate in formula-fed infants compared to exclusively breastfed infants [108]. However, recent studies confirm that the benefit of reduced HIV-1 infection is hampered by higher mortality in formula-fed infants [150]. Studies assessing the risk of HIV-1 transmission by treating the mother with antiretrovirals were conducted in several countries, including Tanzania, Botswana, Malawi and Mozambique. The MITRA-plus study, conducted in Tanzania [151], included breastfeeding mothers who received a combination of zidovudine, lamivudine and nevirapine/nelfinavir from the 34th week to the 6th month post-delivery; the MTCT rate was 5% at 6 months. The DREAM program in Mozambique observed a MTCT rate of 0.6% in a breastfeeding population where mothers received NNRTI-based HAART for 6 months post-delivery [152]. Both studies suggest that further reductions can be made when treating breastfeeding mothers.
To optimize survival of HIV-1-exposed infants, some trials were undertaken to determine whether extended prophylaxis of infants would decrease the rate of HIV-1 transmission. The Post-Exposure Prophylaxis of Infants (PEPI) [153], a large, randomized clinical trial conducted in Malawi, compared extended prophylaxis of infants with nevirapine plus zidovudine for 14 weeks and nevirapine combined with one week of zidovudine (standard in most Sub-Saharan African countries). The PEPI study demonstrated that both extended prophylaxis regimens significantly reduced the risk of post-natal transmission at 14 weeks, with a protective efficacy of more than 60%. However, the cumulative risk of post-natal infection between birth and 14 weeks was 2.8% in the group receiving nevirapine plus zidovudine for 14 weeks and 8.4% in the group receiving nevirapine combined with one week of zidovudine. In Ethiopia, Uganda and India, the Six-Week Extended Nevirapine (SWEN) compared single-dose maternal/infant nevirapine alone to single-dose nevirapine plus extended daily infant nevirapine during 6 weeks and found a 46% decrease in postnatal HIV transmission at 6 weeks.

Thus, observational studies confirmed by randomized clinical trials confirm the efficacy of both treating the breastfeeding mother and the HIV-1-exposed infant during the first 6 months, and there seems to be a similar efficacy of maternal HAART and infant prophylaxis for PMTCT [154]. Data provided to date have confirmed the short-term safety for both mother and infant. Resistance, however, is still a concern, and more studies are needed to assess its real impact.

1.5.2 Horizontal Transmission
While heterosexual transmission remains the primary mode of HIV-1 transmission among adults in Africa, mother-to-child transmission is the main mode of transmission in children. Horizontal transmission (such as needle stick injuries, blood transfusions and scarifications) plays an uncertain role in HIV transmission in resource-constrained and high HIV prevalence settings. The role of horizontal transmission has been controversial, and reports and estimates vary from 5% to 30% in low-resource settings [155-158]. Parenteral transmission is defined as that which occurs outside of the gastrointestinal tract, such as subcutaneous, intravenous, intramuscular and intrasternal injections [158]. The relative percentage of HIV-1 infection caused by each of these routes depends on several factors, including the prevalence of infection...
among particular groups and their shared behaviours [158-161]. Health care providers and traditional healers both in and out of healthcare settings in Africa administer a large number of injections. Thus, parenteral transmission could contribute significantly to HIV-1 infection in the region [156, 158, 161-167]. A study analyzing nosocomial outbreaks in Russia, Romania, China, India, Mexico and Libya estimated that one iatrogenic infection occurred after 8-52 procedures involving HIV-infected persons, although only 0.3% of healthcare workers seroconvert after percutaneous exposure [165]. A case-control study reported that deep injuries and other risk factors collectively increased seroconversion risk by as much as 50 times [168]. It has been estimated that the transmission efficiency in medical settings, with no or grossly insufficient efforts to clean equipment, ranges from 0.5-3% for lower-risk procedures (e.g., intramuscular injections) to 10-20% or more for high-risk procedures [165]. Nosocomial transmission through blood transfusions and needle sticks were the main modes of transmission at the beginning of the epidemics, especially among leukemic patients and drug users, as has been demonstrated in the US [169]. However, other studies have documented blood transfusion as a mode of transmission in Romania [170] and the sub-Saharan region [162, 165], including Zaire [171] and Nigeria [172]. Horizontal transmission of HIV-1 outside the healthcare system is a reality, and there are some reports regarding child-to-child transmission[173], intra-familial transmission [174], human bites [175] or even cosmetic services through tattooing [160] contributing to HIV-1 transmission. Although playing a less important role, nosocomial infections should be completely avoidable, and education and safe medical procedures should always be emphasized to prevent horizontal transmission of HIV.

1.6 Natural History of HIV Infection in Children

HIV-1 infection and disease progression are different in children and adults. The majority of children develop mild symptoms in the first few months of life, similar but not identical to those observed in adults, that disappear after some time [176]. Contrary to what occurs in children, rapidly progressive HIV-1 disease in adults is rare, as is HIV-1-associated encephalopathy [177, 178]. For instance, in Europe, 20% of HIV-1-positive children progress to CDC category C in the first year, with an annual progression of 5% thereafter up to 6 years of age [176], whereas in adults, progression
is gradual in the first 2-3 years after infection, with a steep increase in the following 2-3 years, followed by another, more gradual increase [179].

In the natural history of vertically acquired HIV infection, the age at onset of AIDS is often described as bimodal [99, 180-184]. There are infected children who develop AIDS very early in the first year of life and others who survive years without AIDS [181-183]. In vertically infected and untreated children, approximately 20% progress rapidly to AIDS or death in the first year of life [176]. Disease progression is less rapid between the ages of 1 and 5 years and slow between 5 and 10 years of age [185]. Early reports from the European Collaborative Study (ECS) [81] showed that about a quarter of vertically infected children developed AIDS in the first year of life, and 49% developed AIDS by 4 years of age. In the ECS cohort, HIV-1-infected children had a mortality of 10% in the first year of life and 28% in children under the age of 5 years, although mortality varied between HIV-infected children from different European countries. Long-term non-progressors (LTNP), or HIV-1-infected children who can maintain a high CD4 % and absence of symptoms despite not being administered antiretroviral treatment, represent a small percentage, between 2% and 16%, of those infected with HIV-1 [186]. Some factors have been identified as being predictive of LTNP, such as a strong cellular and/or humoral immune responses to HIV-1 [187], genetic markers such as CCR5 [88, 91] and CX3CR1 polymorphisms [94], SDF-1 [92, 93] and some HLA haplotypes [85].

In sub-Saharan Africa, the bimodal expression of HIV-1 paediatric disease seems similar to that of industrialized countries, but mortality is much higher [188]. Mortality estimates from one meta-analysis point to 35.2% by one year of age and 52.5% by two years of age [189], with fewer than 25% of HIV-infected children reaching the 5th year of age [188]. The documented differences in morbidity and mortality within the US, Europe and Africa are important and need further explanation. This could be partially explained by differences in HIV subtypes, genetic susceptibility, differences in infectious environment and differences in health infrastructure and access to health care across Europe, the US and Africa.

1.6.1 Primary HIV Infection

Primary HIV-1 infections have different clinical, immunologic and virologic characteristics in early and later infections [116, 190-192]. Disease progression in
infants infected perinatally with HIV-1 is influenced by several factors, such as the time of infection and clinical, virologic and immunologic characteristics of the mothers and infants. It has been described that children infected in uterus have an earlier and more severe form of the disease [192]. Advanced maternal HIV disease during pregnancy [190, 193], as well as maternal low CD4 absolute counts [41, 190, 193] or high viral load [112-114, 116], are factors associated with infant disease progression [194].

Factors associated with disease progression at 6 months of age are not the same as those predicting disease at 18 months or later. Maternal low absolute CD4 during pregnancy and infant lower CD4 percentage in the first 2 months, associated with the presence of lymphadenopathy, hepatomegaly and splenomegaly, predict early disease at 6 months, whereas high viral load and clinical progression to CDC category B or C predict AIDS disease progression by 18 months of age [194, 195].

1.6.2 Symptomatic Disease and Paediatric AIDS

The signs and symptoms of HIV-1 infection in children have been included by the Centers for Disease Control (CDC) in Atlanta, into a classification that is widely used in the US and Europe, known as 1994 CDC Clinical Classification of Paediatric HIV infection [196]. This classification is divided into 4 categories, which are mutually exclusive and grouped as follows: N (asymptomatic), A (mild symptoms), B (moderately severe symptoms, including lymphoid interstitial pneumonitis (LIP)) and C (severe symptoms, including serious recurrent bacterial infections, severe opportunistic infections, HIV encephalopathy, wasting syndrome and cancers). In resource-constrained settings, due to a lack of diagnostic facilities, WHO has provided a similar classification, divided into 4 stages, in which stage 1 is asymptomatic and severity progresses from grade 2 to grade 4 [197]. The CDC and WHO have also provided in the same classifications an assessment of paediatric immune status, in which immunological status is graded into three categories according to age group. The categories in the clinical and immunological classifications do not necessarily correlate; however, both classifications are clinically very useful, and the goal is to provide clinicians with a tool to help understand disease severity and prognosis and therefore better support clinical decision-making.
Paediatric AIDS follows a bimodal expression in industrialized countries and resource-constrained countries. However, there are differences in clinical manifestations. African HIV-1-infected infants develop disease early in life and have more severe morbidity and mortality [188, 198-200]. In HIV-1-infected children, the most frequently reported signs and symptoms in the first year of life are lymphadenopathy, splenomegaly and hepatomegaly [178], with studies from resource-constrained settings showing that, unlike in Europe and the US, malnutrition is a common feature in paediatric AIDS in African children of all ages [188, 201-210]. Other frequent signs include oral candidiasis, chronic cough, chronic parotitis, generalized dermatitis, persistent diarrhoea, pulmonary infections, tuberculosis and HIV encephalopathy [176, 185, 188, 199, 206, 211-218]. When included in the initial pattern of HIV disease, malnutrition is associated with an increased risk of death [219-222], as well as *pneumocystis jiroveci* pneumonia [111, 212], whereas the occurrence of lymphoid interstitial pneumonitis is associated with a slower progression of HIV disease [188].

1.6.2.1 Immune status and clinical course

Immune status in HIV-1 disease is often assessed by the number or percentage of circulating CD4+ cells. In infants and children under 6 years of age, the absolute number of CD4+ cells is higher than in adults, declining to reach adult levels by 6 years of age [223]. Nonetheless, the absolute number of CD4+ cells varies greatly with age; therefore, the assessment of CD4 percentage, being independent of age and showing less variability, is preferably used in children younger than 6 years of age. The CD4 assessment has been validated as a good marker of HIV-1 disease progression in children [222, 224] and is a good instrument to assess responses to antiretroviral treatment in adults [225] and children [226]. Immune status, defined by the CD4 absolute count and percentage, can be classified as moderate, severe or absent in a classification that takes the age group into account. The more widely used classifications are those provided by the OMS and CDC. For some resource-constrained countries where CD4 is not largely available, OMS has validated the use of total lymphocyte count (TLC) as a guide to start HAART and monitor patients during treatment. The WHO immune classification includes TLC. The CD4 count assessment is a key element in the HIV-1-infected patient, because its decrease is correlated with higher morbidity and mortality in both adults and children.
The younger the infant, the greater the impact that a low CD4 count can have on mortality, as shown by the European collaborative study [176].

1.6.2.2 Viral load and clinical course
Viral load, or the quantitative assessment of HIV-1 RNA copies per ml, has been validated in the monitoring of adults [227] and children [41, 226, 228]. Acute infection by HIV-1 in adults is translated into the appearance of high levels of HIV-1 RNA in plasma and serum [229]. These high levels of HIV-1 RNA start decaying after 2 months [229]. In children, however, there are high levels of HIV-1 RNA present in the plasma and serum at the age of 1.5-3 months [40, 42], which start decreasing to reach minimal levels at 5-6 years of age [40, 42, 230]. The high viral load in infants has been related to HIV replication within rapidly proliferating target cells in a developing immune system [231],[232].

In HIV-1-infected adults, there is a good correlation between viral load and the risk of progression to AIDS and death [227]. In children, the viral load has also been confirmed to be a good predictor of disease progression [42, 228, 233]. Viral load assessment is also a good instrument to monitor adults [225] and children undergoing ART [234, 235].

1.6.2.3 Opportunistic infections
Opportunistic infections (OI) in HIV-infected patients are known to occur with immunossupression, and prevention and management of OI are key components in the management and care of HIV-1-infected children. OI constitutes the primary cause of death in HIV-1-infected children prior to HAART in resource-rich settings and continue to be primary causes of death in HIV-1-infected children in resource-constrained settings [211]. The prevalence of OI in the absence of HAART depends on a number of factors, such as the child’s age, immune status, previous OI and the pathogen agent [236]. HAART, by suppressing viral replication and allowing for immune reconstitution, results in less OI [237]. Studies in adults and children undergoing HAART have shown a significant decrease in OI incidence and related deaths [237]. In the US, the incidences of OI, as described by PACTG [238], have greatly changed in the periods from 1988-1998 and 2000-2004, pre- and post-HAART, respectively. For instance, the incidence of bacterial pneumonia decreased from 11.1
per to 2.2 per 100 child-years, bacteraemia decreased from 3.3 to 0.4 per 100 child-years, and PCP decreased from 1.3 to 0.09 per 100 child-years [238]. In Thailand, the rate of severe opportunistic infections leading to hospitalization dramatically reduced after the introduction of HAART, as the hospitalization rate decreased from 30.7% during the first 24-week period to 2.0% during weeks 120-144 after the initiation of HAART [209].

Various pathogens can cause OI, such as bacteria, virus, fungi or protozoa, but while bacterial infections are treatable, some protozoans, fungal or viral OI are not curable with available treatments. One example is cryptosporidiosis or microsporidiosis, for which no specific treatment is available; however, with efficient HAART, it can be improved. OI prophylaxis is crucial in the management of HIV-1-infected children, especially those with immunosuppression, yet attention must be paid when initiating HAART in those with severe immunosuppression, as it can lead to Immune reconstitution inflammatory syndrome (IRIS). Patients who are severely immunosuppressed with an acute or latent OI at the beginning of HAART are prone to an exaggerated inflammatory reaction that can clinically worsen the disease and sometimes threaten the patient’s life.

In HIV-infected children on HAART, OI can occur due to suboptimal medical care, treatment failure due to lack of adherence, drug resistance, drug-drug interactions or an IRIS. In addition, as immune function deteriorates the tendency to suffer from OI increases. The natural history of OI in children differs from that in adults. In adults, OI are secondary to the reactivation of pathogens that were often acquired before HIV infection, when the patients were immunocompetent. In children, however, OI often occur as primary infections. In perinatally-infected children, OI occur after HIV infection is established and at times when immune function is already compromised. This makes OI manifestations in children different from those of adults, e.g., a young child with tuberculosis is more likely than an adult to have disseminated and non-pulmonary infection. Children are also more vulnerable, as when they acquire the HIV through MTCT, they also frequently acquire other opportunistic pathogens, depending on the mother’s disease state. In households, other family members are also frequently sick, thus exposing the children to a number of pathogens.
1.6.2.4 Growth and HIV-1 infection

At a time when the HIV/AIDS pandemic has attained its highest prevalence in Sub-Saharan Africa, malnutrition affects more than 555 million children worldwide, especially in south-central Asia and sub-Saharan Africa, with 32% of children stunted, 3.5% wasted and 20.2% underweight [239]. AIDS and malnutrition are intimately related, and before the introduction of antiretroviral therapies in Africa, a number of studies from Uganda, Malawi, South Africa and Rwanda showed that HIV infection in children is associated with poor growth, stunting and wasting [240] [206, 241, 242]. Higher rates of HIV infection have also been documented in malnourished children from Tanzania [243], Burkina Faso [220] and Uganda [220].

AIDS-associated malnutrition has been associated with infections, abnormal metabolic functions, abnormal energy expenditure, food scarcity, multiple parasite infestations, gastrointestinal malabsorption and psychosocial problems [244, 245] [246], [247-249] [250]. AIDS-associated malnutrition has been used as a marker of advanced disease stage, and studies performed on Tanzanian adults and Ugandan children have shown malnutrition to be a predictor of mortality and poor immunological response [240, 252]. A recent study from Zambia [253] also demonstrated that HIV-infected children with severe malnutrition respond well to nutritional rehabilitation, although nearly all require early ART to prevent disease progression.

The introduction and scale-up of ART in African children have demonstrated an overall improvement in growth and immune reconstitution [245, 254] [201, 210]. As a result, it has been suggested that in resource-constrained settings, weight improvement could be used as a default marker of good ART response [255]. Several disturbed growth patterns have been described in HIV-infected children [248, 255-257]. Nearly all are consistent that HIV-vertically infected children have their growth affected early and are usually shorter than uninfected children [258]. Growth has, however, been shown to be heterogeneous in patients undergoing ART, with some studies demonstrating significant improvements in weight and height without an improvement in body mass index [259], or an improvement on muscle mass but no improvement in height [250] [260].

A better understanding of growth patterns in HIV-infected children can allow adequate nutritional interventions that can help reduce morbidity and mortality, improving medical outcomes and quality of life. In this context, it is also important to
understand the impact of malnutrition on the growth of children undergoing ART, as has been suggested by a study in India [261] [221] that in HIV-infected children, pre-existing malnutrition may impair the nutritional response to ART. In addition, ART response and growth parameters in African children with pre-existing malnutrition have not yet been studied.

1.6.2.5 Neoplasias and Kaposi’s sarcoma

Cancers are known to occur in surplus in AIDS patients. It appears that children with AIDS are at lower risk of developing cancer compared to adults [262].

Data regarding HIV-1-infected children with cancers are scarce and based on series reports, but a population-based study in the US [262] showed that the spectrum of AIDS-associated paediatric cancers resembled that seen in adults, with the addition of leiomyosarcoma. In the same study, both primary brain lymphomas and leiomyosarcomas tended to occur in children surviving several years after AIDS onset. The usually frequent tumours in AIDS patients are non-Hodgkin lymphomas and Kaposi’s sarcoma. In a study from Malawi [263] reporting on the spectrum and presentation of paediatric malignancies associated with HIV, the most frequent tumours were Burkitt’s lymphoma, retinoblastoma and Kaposi’s sarcoma (KS), in 50%, 13% and 9% of cases, respectively. In the same study, the authors observed an increase in the frequency of KS cases that was greater than that seen for other cancers, and in recent years, KS has emerged as the second most frequent childhood cancer in Malawi.

KS has been shown to be endemic in sub-Saharan Africa for many decades [264, 265]. With the spread of HIV infection, a new form of AIDS-associated KS (AKS) has emerged [266]. Kaposi sarcoma herpes virus (KSHV), also called human herpes virus-8 (HHV8), is the etiological cause of all forms of KS [266]. The seroprevalence of HHV8 in endemic countries (Italy, Greece and countries in the Central and Southern East parts of Africa) varies between 10-70% in the adult population [267]. HHV8 seroprevalence rates in children tend to reflect those observed in adults, albeit at lower levels, with steady increases from early childhood, reaching near adult prevalence before puberty [267]. It has been estimated that in Europe and the United States, the risk of KS in HIV-infected people is increased as much as 1000-5000 times compared to the general population, whereas in Africa, this risk is increased approximately 30-50 times [265].
Sub-Saharan Africa is home to the majority of HIV-1-infected patients as well as those infected with HHV8, and it was estimated that, in 2002, there were 39,500 males and 17,100 females with KS in sub-Saharan Africa [265]. In adults, transmission is mainly sexual [268],[269, 270]. In children, transmission from mother to child has been demonstrated [271], although it is unlikely to occur through breastfeeding, since the virus could not be isolated in milk. Horizontal transmission of the virus within families has been reported in a few studies [264],[272-274]. There are different forms of KS in adult and children, and endemic KS (EKS) in children is predominantly lymph glandular with or without skin involvement [275, 276]. EKS in children tends to affect males preferentially, and the high-risk group is children 0-5 years old [264, 276]. AKS has been essentially described for the adult population. Treatment in adults in resource-rich settings includes HAART and chemotherapy. Treatment options can be very toxic or technologically demanding, although recently, pegylated liposomal doxorubicin and liposomal daunorubicin have proven to be simpler, effective and well tolerated [277]. However, these therapies are unlikely to be available or affordable in many resource-poor settings, where the bulk of HIV infections and KS occur. To date, there are few studies reporting on the effectiveness of HAART in adult patients with AKS [278-280].

An alternative treatment is the sequential use of HAART followed by chemotherapeutic agents if KS regression is inadequate (26). However, there is a concern that overlapping toxicities and pharmacokinetic interactions may affect the therapeutic effectiveness of HAART and chemotherapy. There is no information concerning the use of HAART and chemotherapy in AKS in African children.

### 1.6.3 Diagnosis of HIV Infection

#### 1.6.3.1 Clinical

Diagnosis, especially early diagnosis, is crucial for HIV-1-infected children, as it allows better care and management, initiation of early therapy and thus a better prognosis and quality of life for the HIV-1-infected child.

Clinical diagnosis is based on clinical suspicion confirmed with laboratory tests. Once the diagnosis of HIV-1 infection is confirmed, the child is then classified according to either the CDC or WHO clinical classification, for a better assessment of disease severity.
Laboratory tests for HIV-1 infection in children rely on the detection of antibodies or viral particles. Testing for HIV-1 antibodies is of little value in children who are vertically infected and under the age of 18 months, due to the transfer of maternal IgG anti-HIV across the placenta. These antibodies usually disappear by 9-10 months of age [81], although in some cases, they last up to 18 months [281]. Other antibodies, such as IgA and IgM anti-HIV, have proven useful in confirming infection in infants older than 4 months [282]. The most commonly used methods for detection of HIV antibodies are enzyme-linked immunosorbent assays (ELISA) and various types of rapid simple assays [283]. These assays are confirmed by immunoblot assays, usually Western blot or line immunoblot, in resource-rich countries. Due to the high cost of immunoblot assays in resource-limited settings, there are cheaper strategies that include a combination of two or three rapid tests with ELISA.

The detection of an HIV antigen, such as the protein p24, has also proven useful in confirming HIV-1 infection in infants and children [282, 284, 285]. Virus culture of HIV-1 is a reliable test to confirm early infection [283], but due to its high cost, it has not been widely used.

Finally, the most used and useful methods for identifying paediatric HIV infection in clinical practice are gene amplification techniques for detection of either RNA or DNA of HIV by the polymerase chain reaction (PCR) method. Detection of HIV RNA is a quantitative method in which the number of copies is measured, whereas HIV DNA detection is a qualitative method that is performed in peripheral blood mononuclear cells [284]. Both tests are highly sensitive and specific [284], though these specificities and sensitivities change with the age of the infant. In a study conducted in HIV-1-exposed infants [286], HIV-1 DNA PCR had a sensitivity of 27% in infants less than 3 weeks of age, while qualitative and quantitative RNA PCR had a sensitivity of 64%. Each assay had a sensitivity of 96.2% at 4-6 weeks and 100% at more than 7 weeks of age. Specificity of HIV-1 DNA PCR for all age groups was 100%, but specificities of qualitative and quantitative RNA PCR assay were 96.1% and 95.5%, respectively. This means that HIV-1 RNA PCR could offer a slight benefit in sensitivity over DNA PCR in the diagnosis of HIV infection in young infants. Positive RNA results can be found in a
small number of infants who are not infected with HIV-1. HIV-1 RNA detection should not be routinely used in isolation for the diagnosis of HIV infection in young infants [286]. In resource-rich settings, the early diagnosis strategy includes 3 HIV DNA PCR tests, the first at birth, the second at the first month and the third in the 4-6-month period [287, 288].

Due to the cost of these tests, however, in resource-constrained settings, the early diagnosis strategy is different and varies within settings, but WHO recommendations suggest the use of a virologic test at 4-6 weeks, which is confirmed at 9-18 months with a rapid test [197].

1.6.4 Paediatric Treatment

Paediatric care and management of HIV-1-infected children include a combination of strategies to improve survival and allow a better quality of life for these children. Antiretroviral treatment has completely changed the scenario of the management of HIV-1-infected patients. However, there are other measures that, in addition to ART, improve the prognosis of HIV-1-infected children, including immunizations, nutrient supplementation, prevention and treatment of opportunistic infections, emotional support, pain relief and palliative care.

1.6.4.1 Nutritional interventions

Nutritional interventions include nutritional counselling and nutritional supplementation. Nutrition counselling has shown to result in increased energy intake by HIV-infected patients [289]. Micronutrient deficiencies and malnutrition are common features in HIV-1-infected children, and nutritional supplementation is essential for survival and better treatment response in HIV-infected patients [263, 290]. Micronutrient supplementation has been implemented using a micronutrient mixture in a nutritional supplement or through vitamin A supplementation. Vitamin A, in quarterly supplements in HIV-infected children in Tanzania, has resulted in a 63% decrease in all-cause mortality and a 92% decrease of diarrhoea-related mortality [291]. In South Africa, vitamin A supplementation for HIV-infected children has resulted in a 31% reduction in mortality and a 49% reduction in diarrhoea incidence [292].
1.6.4.2 Prevention and treatment of infections

Endemic infections, such as helmintic infections, have been related to higher HIV viral loads in Africa. A study performed in Ethiopia showed that viral load correlated with egg burden and the number of helmintic infections [293] [294]. In addition, the provision of regular anti-helmintic treatments have been shown to decrease diarrhoea incidence by 30-50% at the district level in Zambia [295]. Malaria is the leading cause of morbidity and mortality in Africa. Studies performed in adults have shown that viral loads can be 7 times higher in HIV-infected patients and can be successfully reduced with antimalarial treatment [296]. In addition, lower CD4 counts are associated with an increased frequency of malaria as well as with higher malaria parasitemia in Ugandan adults [297].

OI are common in HIV-1-infected children, and their frequencies are higher with a decrease in immune status. There are a wide variety of OI, but in this context, only the more frequent and preventable will be discussed. Pneumonia caused by *Pneumocystis jiroveci* (PCP, former *carinii*) is a major cause of morbidity and mortality in HIV-1-infected infants, and its case-fatality rate (40-87%) and peak incidence (3-6 months of age) is similar in both resource-rich and resource-poor countries [298], [299], [300], [301], [302], [303]. PCP disease is preventable with cotrimoxazole. Prophylaxis with cotrimoxazole has resulted in a 47% decrease in morbidity and mortality in Zambia [304]. Cotrimoxazole is also effective in preventing *Toxoplasma gondii* infection, *Streptococcus pneumoniae*, *Salmonella*, *Nocardia*, *Isospora belli* and *Plasmodium falciparum* infections [305]. However, the widespread use of cotrimoxazole has the potential to increase antibiotic resistance to *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Enterobacteriaceae* and eventually *Plasmodium falciparum* [306], [307], [308]. Tuberculosis (TB) is one of the most common infections in HIV-infected patients [309] and is a cause of high mortality in HIV-1-infected children [310]. The incidence of tuberculosis in neonates and infants has doubled due to the increased incidence in pregnant women [311]. Children in close contact with adults with TB have an 80% risk of becoming infected, with up to 52% developing active TB [312]. The drug isoniazid, used for TB prophylaxis, has shown an early survival benefit and reduction in incidence of tuberculosis in children with HIV [313], supporting its use in the management of HIV-infected children.
1.6.4.3 **Psychosocial support**

The grief of pain, abandon, stigma, depression and other psychological factors have negative impacts on the ways patients react to disease. In this context, psychosocial support plays a critical role in the management of HIV-infected children and their families, helping the patient and his or her family cope with HIV disease and its consequences. The relief of pain is also extremely important, as severe pain is common in HIV-infected children [314]. There has been little research regarding psychosocial support for HIV-infected children in Africa. However, play therapy, coloured pictures on hospital walls and love and attention can contribute to the well being of sick children [200].

1.6.4.4 **Immunizations**

Immunizations are essential for children’s health promotion and, in particular, for HIV-1-infected children. Vulnerability to severe, recurrent or unusual infections by vaccine-preventable pathogens is increased in HIV-1-infected children. Invasive pneumococcal disease is 40 times more frequent amongst HIV-infected compared to uninfected children in South Africa [315]. In the US and Europe, pneumococcal disease is 5-10 times more frequent in HIV-infected compared to uninfected children [316]. With few exceptions, vaccines are safe in the context of HIV infection, but their efficacies are somehow impaired in these children. Defects in antigen-presenting-cell, B-lymphocyte and T-lymphocyte responses may negatively impact the vaccine response [317]. Some HIV-infected children show lower levels of protection following immunization due to defects in the generation of immunologic memory or clonal deletion of memory T and B cells [318]. In HIV-1 symptomatic children, the administration of live attenuated or viral vaccines is not recommended [317]; in these children, deferment of Bacillus Calmette-Guerin and yellow fever vaccines has been recommended [319]. Some vaccines have the potential to provide additional protection in HIV-1 infected children, such as pneumococcal vaccine and the vaccine against *Haemophilus influenzae*. The 9-valent pneumococcal conjugate vaccine has prevented 65% of invasive pneumococcal disease in HIV-infected children in South Africa [320]. Some vaccines lose efficacy over time in HIV-infected children, and it has been shown that an additional measles vaccine should be administered at 6 months of age [319]. A lower efficacy (44%) of the *Haemophilus influenza* vaccine has been shown in HIV-infected children [321, 322].
However, the *Haemophilus influenza* type B (HiB) conjugate vaccine was effective in preventing invasive HiB disease in African HIV-1-infected children [322, 323]. Diphtheria, tetanus and pertussis immunizations have been shown to give suboptimal immunogenicity and immunogenicity of shorter duration [317] in HIV-infected children. However, following HAART initiation, boosting immunization reconstitutes immune responses [317]. In a study conducted in HIV-infected children undergoing HAART, to assess cellular and humoral immune responses to tetanus toxoid booster, the majority of patients developed protective antibodies after the booster and maintained the response [324].

1.6.4.5 *Antiretroviral treatment*

Antiretroviral treatment is based on a combination of antiretroviral drugs and has been associated with a dramatic reduction in morbidity and mortality of adults and children in rich and poor countries. There are 22 antiretroviral drugs currently approved by the United States Food and Drug Administration for use in the treatment of HIV-infected adults, but only 12 of these drugs are approved for use in children [325]. Antiretrovirals belong to six major classes: nucleoside and nucleotide analogue reverse transcriptase inhibitors (NRTIs), non-nucleoside analogue reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), fusion inhibitors, integrase inhibitors and co-receptor binding inhibitors. According to guidelines developed by international organizations such as the WHO, the Paediatric European Network for treatment of AIDS (PENTA) and the National Institutes of Health (NIH) in the United States, the treatment of choice for ART-naïve and HIV-infected children is based on a combination of 3 drugs, 2 of which are NRTIs and the third is either a NNRTI or a PI. The paediatric antiretroviral treatment poses specific challenges, such as the need for a larger number of and better paediatric formulations, the poor availability of pharmacokinetic data for children, the potency and toxicity of the drug regimen, the psychosocial environment of the child, previous exposure to antiretroviral drugs in the PMTCT and a major lack of skilled staff in poor countries. Highly active antiretroviral therapy (HAART) is currently recommended for the initial treatment of HIV-infected children, as it allows for better survival through immune restoration and control of disease progression [176, 201, 326-329].
1.6.4.5.1  When to start

The question of when to start is very important in the treatment of HIV-infected patients, as it differs according to patient age and availability of resources. In resource-rich settings, there is an individualized approach, whereas in resource-constrained settings, a public approach is recommended, in which individual needs remain invisible. In adults, observational studies indicate that HAART started at a CD4 absolute count of 200-350 cells/mL results in better immune reconstitution and lower disease progression [330],[331],[332]. The choice to start early therapy has advantages, such as viral replication control, preservation of immune status and prevention of clinical disease progression. Starting later or delaying HAART until clinical and immunological symptoms appear has the benefits of better adherence in a symptomatic patient, less toxicity and eventually reduced evolution of drug-resistant virus [325]. In HIV-infected infants, because of high mortality rates of 30-35% [184, 189] during the first year of life and the demonstrated benefits of early HAART [329, 333, 334], the recommendations are to treat all, regardless of clinical or immunological status [222, 224, 226, 329, 335]. Good clinical and immunological outcomes have been described for early treatment [329, 333, 336, 337], but the concern is related to incomplete viral suppression (30-70%) and the emergence of drug-resistant virus. In older and symptomatic children, there is a general consensus in the different guidelines to start HAART in stage B or C (CDC classification) or stage 3 and 4 (WHO classification) and with CD4 percentage less than 25% [196, 197]. However, children who survive infancy without major HIV-related morbidity have a better capacity to manage the virus [338] and thus remain asymptomatic or mildly symptomatic. In the case of mild symptoms with good immunological status, the decision to start HAART is poorly supported by the available data [197, 234, 235].

1.6.4.5.2  What to start

The initiation of a combination antiretroviral therapy with at least three drugs from two different drug classes is recommended in HIV-1-infected infants and children [197]. The NIH [235], PENTA [234] and WHO [197] guidelines recommend a NNRTI-based or protease inhibitor-based HAART as a first-line treatment in ART-naive patients. However, in the case of previous drug exposure through PMTCT, modifications must be made accordingly. WHO recommends the use of ritonavir-
boosted lopinavir in cases of HIV-infected infants with previous exposure to nevirapine, due to the risk of nevirapine resistance. Nevirapine has one of the lowest genetic barriers; one mutation is sufficient to confer resistance, which is the main reason it has not been included in first-line treatments in resource-rich settings. However, in resource-poor settings, nevirapine is the drug of choice for first-line treatment due to its low cost and the fact that it is included in most fixed-dose combination antiretroviral tablets that are widely used for massive ART scale-up in poor countries.

1.6.5 Resistance to paediatric antiretrovirals

Resistance to ART has become increasingly important, as the use of ART is becoming more prolonged. Resistance to HIV occurs due to mutations in the viral genome that allow viral resistance to ARV drugs. Because HIV is a virus with a high replication rate, high error rate of HIV-1 reverse transcriptase and rapid viral turnover, its ability to mutate is also high [339]. These features allow the introduction of many mutations in the viral population that may confer drug resistance.

HIV resistance to ART can be divided into 2 categories: primary resistance, which reflects the acquisition of a drug-resistant strain of HIV by a newly infected patient, and secondary, or acquired resistance, which develops in a patient undergoing ART [340]. Viral replication during periods of drug exposure may result in the selection of viral strains resistant to one or more drugs exerting a selective pressure [339, 341]. The selection of viruses resistant to any drug in a combined treatment regimen depends on the selective pressure exerted by the drug, the genetic barrier to resistance (i.e., the number of genetic mutations required to cause ARV resistance), the virus subtype and the cost of resistance to that virus [341]. From the patient side, adherence plays a critical role in the development of resistance. Resistance can develop to one or more classes of drugs, especially when adherence is sub-optimal [197]. Adherence and resistance relationships are class-specific [342], as different drug classes have different resistance rates at different adherence levels. Resistance to boosted protease inhibitors is uncommon at all adherence levels [342], but a high risk exists in individuals with 75-95% adherence [343]. Resistance to NNRTI is more likely at lower levels of adherence [342], and a study in Italy has shown that the risk of failing therapy with NNRTI resistance was 3.5 times higher in individuals with 75-95%
adherence compared to 5 times higher in patients with less than 75% adherence [343]. Overall, resistance is more common for NNRTI and deoxycytidine analogue NRTI, followed by non-boosted protease inhibitors. Boosted protease inhibitors are the least prone to resistance [342].

Currently, HAART in drug-naïve patients consists of a combination of two NRTI with one NNRTI or PI. The emergence of drug resistance in patients receiving first-line HAART, especially NNRTI-based, is associated with increased mortality [344]. Some studies have now shown that genotypic resistance is more frequently detected in patients who are on HAART regimens that include NNRTI compared to HAART regimens that include PI [345-347].

Most studies investigating the genetic mechanisms of HIV-1 drug resistance were carried out on HIV-1 subtype B viruses, which account for 12% of the HIV pandemic in the world [340]. The differences among subtypes can impact the number and type of mutations developing under selective pressure [348]. Nevertheless, the massive scale-up of HAART is occurring in regions of the world where non-B types are circulating and where poor health conditions can theoretically contribute to the emergence of drug resistance. A study conducted in South Africa [349] has, however, shown similar mutations among adults and children, and these mutations were comparable to those found in subtype B-infected patients.

The pathogenesis and mechanisms of resistance differ within drug classes. Resistance to different NRTI drugs, an important class of ARV, can result in different amino acid substitutions [350]. Some of these mutations have been shown to cause cross-resistance or multiple resistance among various drugs of this class [351]. By contrast, some combinations of amino acid substitutions can lead to increased drug susceptibility and may also result in re-sensitization of formerly resistant viruses. The mechanism of resistance to zidovudine is primer unblocking (i.e., nucleotide excision) [351]. The rate of nucleotide excision by reverse transcriptase (RT) can be increased by thymidine analogue resistance mutations (TAMs) [350]. By contrast, RT mutations that sensitize HIV-1 to zidovudine (e.g., M184V and Y181C) do so by slowing the rate of excision. M184V is a mutation that confers high resistance to lamivudine (an NRTI drug); however, this mutation has the capacity to significantly reduce viral replication and lower CD4 cell decline [350, 352] and to delay or prevent the emergence of TAMs
Resistance to NNRTI drugs is accounted for by the most commonly observed mutations, K103N, V106A/M, V108I, Y181C and G190A/S. NNRTI resistance is important because of the widespread use of nevirapine in PMTCT programs across poor countries. Studies assessing resistance following single-dose nevirapine for PMTCT have shown NNRTI resistance mutations in about 65% of women's plasma samples at 6-36 weeks after exposure [354]. This type of resistance seems to be higher for women infected with HIV-1 subtype C compared to subtypes A and D [355]. These mutations, however, fade in a period from 12-24 months [134]. Children exposed to nevirapine through PMTCT programs also present resistant mutations, but a study conducted in Uganda demonstrated that treatment outcomes with nevirapine-containing HAART were not affected by these mutations [356]. The K65R mutation is the main mutation selected by tenofovir; it confers cross-resistance to several other NRTI drugs, including abacavir, lamivudine, didanosine and possibly stavudine but also sensitizes the virus to zidovudine [350].

Studies performed in the UK show that children in the UK cohort with ARV resistance increased from 6% in 1998 to 23% in 2004, but the percentage of samples containing at least one major mutation remained constant, approximately 70% for NRTI, 60% for NNRTI and 30% for PI drugs [296, 357].

Ideally, patients should not remain on failing regimens for prolonged periods, as this allows the emergence of further resistance and complicates future regimens.
2 Study Aims

Paediatric antiretroviral treatment has recently been massively scaled up in Africa. Results from paediatric treatment are important to bring additional evidence of efficacy and also to point out difficulties, challenges and major gaps in knowledge. We aimed to describe the main characteristics of paediatric HIV infection and treatment outcomes in one of the countries most strongly affected by the HIV pandemic as well as one of the poorest countries on the planet.

2.1 General Objective

To describe epidemiological, clinical and laboratory aspects of paediatric HIV infection in a cohort of HIV-1-infected children at the main reference paediatric HIV/AIDS treatment centre in Mozambique, the Paediatric Day Hospital (PDH) in Maputo.

2.2 Specific Objectives

a. To assess risk factors for horizontal transmission in HIV-1-infected children born to HIV-1-seronegative mothers.

b. To describe antiretroviral treatment (ART) outcome in the cohort of HIV-1-infected children attending the PDH.

c. To evaluate the resistance profile to paediatric antiretroviral drugs of children on ART.

d. To describe the outcome of ART and chemotherapy in AIDS-associated Kaposi’s sarcoma.
3 Patients and Methods

3.1 Setting
The patients chosen were those undergoing routine follow-up and care at the PDH at Maputo Central Hospital, which is the main reference centre for the care and management of HIV-infected children in Mozambique. The centre is located at the main teaching hospital in Maputo, the capital of the country. It began its activities in May 1994 as a dedicated HIV consultation and was transformed into a Day Hospital in 2000. Antiretroviral treatment has been available since December 2003.

3.2 Service Delivery Model and Clinical Protocols
The centre provides a comprehensive service for all HIV-infected children and those exposed to HIV, both prior to ART eligibility and once on ART. Care is provided by a team comprising 2 paediatricians, 3 nurses, 2 psychologists, 5 activists and 2 data clerks.

All activities, including medical consultations, laboratory tests and treatments, are provided free of charge. Patient preparation consists of at least 2 counselling sessions, nomination of a caregiver and adherence aid. The adherence model is a patient-centred model, focused on ensuring that the caregiver has a good understanding of the intervention. Adherence is assessed through a combination of pharmacy and clinical records. Pharmacy records dates of drugs picked up, and on clinical files, there is a record based on patient and/or caregiver adherence self-report. Adherence is systematically reinforced by the psychologists and the home-based care team, in case of any suspected fail. Eligibility criteria for ART prior to 2008 were: children at WHO clinical stages III or IV or with CD4 counts below 20% and 15% according to age, below or above 18 months, respectively. The regimens are sequential and are administered according to national guidelines, adapted from WHO guidelines. There are only two lines of antiretroviral treatment, first- and second-line. First-line treatment is based on a combination of zidovudine or stavudine with lamivudine and nevirapine or efavirenz. First-line treatment is, for the majority of patients, a single tablet with a fixed-dose combination of stavudine, lamivudine and nevirapine. This fixed-dose combination exists in dispersible tablets, which are very useful for small children and illiterate caregivers, as it is a good substitute for oral suspensions. This formulation is widely used in resource-constrained settings and has allowed a massive scale-up of paediatric
treatment. Second-line treatment is based on an association of didanosine, abacavir and lopinavir that is ritonavir-boosted. Criteria to switch to second-line treatment are strict and are based on clinical and immunological failure in the presence of good adherence. In 2009, virological failure was included in the criteria to switch the treatment line. If viral load is greater than 5,000 copies/mL in two different measures, a treatment change is considered.

3.3 Clinical and Laboratory Assessment

All children had a systematic interview, physical examination and laboratory assessment at inclusion. Demographic information concerning place of living, parental HIV serostatus or parental ART condition were recorded. Weight, height and cranial circumference were measured at every visit. Two experienced nurses, always using the same instruments, performed the anthropometric measurements. A routine laboratorial assessment, including a haemogram, kidney and liver functions and CD4 counts and percentage were performed at the first month and every six months after starting ART. The CD4 assessment was performed by flow-cytometry using the FACS Calibur, Becton Dickinson, San Jose, CA, USA). There was no routine viral load prior to June 2009. However, in 2006, a cross-sectional viral load assessment was carried out using the Amplicor HIV-1 RNA Monitor v1.5 (Roche Diagnostic Systems, Branchburg, NJ, USA). The detection threshold of the HIV-1 RNA assay was 50 copies/mL. Virological failure was defined as an HIV-1 RNA concentration greater than 50 copies/mL. There was no routine resistance testing. However, in 2007, in those children who had virological failure and had received ART for a period greater than 6 months, genotyping was performed at the virology laboratory in Necker hospital in Paris, France. The genotype tests were performed by the consensus technique of the Agence Nationale de Recherche Scientifique (ANRS) resistance study group. In addition, resistant viruses were defined according to the ANRS resistance algorithm (www.hivfrenchresistance.org). The genotyping assay was validated by ANRS quality control.

3.4 Data Management

Data were collected prospectively on paper forms defined by the Mozambique Ministry of Health (MISAU), such as clinical protocols and pharmacy records, and computerised. Data were then exported to Excel and statistics software for analysis. For the present studies, we used SAS statistical software (version 9.1, Institute Inc.,
Cary, NC) and STATA version 10.0 (Statacorp, College Station, TX). To date, key baseline data are complete. Laboratory results (CD4 counts) and anthropometric measurements (weight, height and cranial perimeter) were complete for 6 and 12 monthly follow-up durations in approximately 80% of individuals. Currently, the vital status of patients is unknown in less than 10% of patients.

Patients on ART who miss medicine collection for more than one month are contacted and/or tracked through home visits where possible, and if 90 days have elapsed since a patient was last seen at the clinic pharmacy, they are considered lost-to-follow-up. Because of the size and rate of the scale-up of antiretroviral treatment delivery, while we collected a great deal of data in patient records, there is a backlog of data awaiting entry into our clinical management software.

Data for patients followed up whilst not yet eligible for ART are available only in paper forms. The length of follow-up on ART was extended to 5 years for some patients. Data were comprehensively validated annually.

Paper I.

Paper I is based on a case-control study, where cases were all HIV-1-infected children born to HIV-negative mothers, and controls were HIV-1-infected children born to HIV-infected mothers during the period of May 1994 to December 2005. We included 22 cases and 77 controls matched for age and gender. The assessed risk factors for horizontal transmission included blood transfusion, injections, surgery, scarifications, dental extractions and sexual abuse.

Paper II.

Paper II is based on a retrospective cohort study describing the main outcomes of antiretroviral treatment in the cohort during the period of December 2003 to December 2008. All 1125 children who started ART during that period were included. Analysis included survival, changes in median anthropometric measurements, changes in haemoglobin, albumin and CD4 percentage, drug tolerance and adherence.

Paper III.

Paper III is based on a cross-sectional assessment of frequency and profile of resistant viruses in those children in the cohort who were receiving ART for at least 6 months and presented with virological failure. A cross-sectional assessment of viral load that was performed in 2006 revealed 135 children in virological failure out of 512 children
on ART for a period greater than 6 months. Genotyping was performed on 84 available samples of the 135 children.

**Paper IV.**

Paper IV is based on an observational cohort of HIV-1-infected children who presented with Kaposi’s sarcoma during the period from December 2003 to December 2008. There were a total of 32 children presenting with biopsy-proven AIDS-associated Kaposi’s sarcoma. Four presented at a very late stage, before any treatment could be performed, and died soon after admission. We included 28 children with AKS who received ART and chemotherapy. Analysis included survival, treatment tolerance and treatment response.
4 Statistics

**Paper I.** Analysis was performed using STATA10 software: median and interquartile ranges for numerical values, odds ratios and 95% confidence intervals for categorical variables, comparisons of categorical variables with Pearson χ² and multiple logistic regressions to assess for relationships between risk factors and horizontal transmission. All reported $p$ values are 2-sided, and $p < 0.05$ was used to determine statistical significance.

**Paper II.** Data were analyzed using SAS version 9.1 (SAS Institute Inc., Cary, NC). Patients lost to follow-up or a change in service provider were censored at the time of the event, while patients alive and in care were censored at the time of the last clinic visit through December 31, 2008. Date of death was taken as the endpoint for those who died. Changes in median CD4 % were compared by a Student’s t-test. Weight-for-age Z-scores (WAZ) and Height-for-age Z-scores (HAZ) over time were compared using the Kruskal-Wallis test. WAZ and HAZ scores were calculated by Epi-Info version 3.3.2, based on the Centers for Disease Control (CDC) standardized weight and height curves. Variables that were statistically significant ($p <0.05$) in univariate analysis were subsequently tested in multivariate analysis. Kaplan-Meier survival methods were used to estimate the probability of survival from the time of initiation of antiretroviral therapy to death.

**Paper III.** Data were analyzed using SAS version 9.1 (SAS Institute Inc., Cary, NC). Association with extended-spectrum resistance was studied using univariate and multivariate logistic regressions. Models were adjusted for sex, age, haemoglobin level and CD4 percentage at the start of antiretroviral treatment, viral load at the time of sampling and duration of antiretroviral treatment. All reported $p$ values are 2-sided, and $p <0.05$ was used to determine statistical significance.

**Paper IV.** The following analyses were performed using *statistica* software: means and standard deviations for numerical values, odds ratios and 95% confidence intervals for categorical variables. Overall survival was calculated from the day of Kaposi’s sarcoma diagnosis until death or 6 months after the end of chemotherapy. The Mann-Whitney test was used for nonparametric continuous variables. All reported $p$ values are 2-sided, and $p <0.05$ was used to determine statistical significance.
5 Ethics

The National Committee on Bioethics Research, the Ministry of Health of Mozambique and the Ethics Committee at Maputo Central Hospital have approved all studies included in this thesis, with references IRB0000257, 1586/APS-5/GMS/2007 and 04/05/2000, respectively.
6 Results and Discussion

Horizontal transmission (paper I)

The risk factors for HIV infection assessed in this case control study were blood transfusions, scarifications, injections, dental extractions, surgery and sexual abuse. Twelve of 22 cases had received at least one blood transfusion, compared to 24/78 controls (p=0.036). This was the only assessed risk factor that was significantly associated with horizontal transmission of HIV in these children. Of the other risk factors, injections were close to significance, with a p-value of 0.09 (21/22 versus 71/78), while the others were non-significant (scarifications: p=1.0; surgery: p=0.5; dental extractions: p=0.5). In 10 children, none of these potential sources of nosocomial or accidental sources of HIV exposure could be identified, although all of these children had previously been in contact with the health services. There is always a residual risk of transfusion-acquired HIV infection despite adequate testing. This is unknown for Mozambique but is especially evident in high-incidence countries [172, 358] The risk is mainly due to the window period before seroconversion. Screening with HIV antigen and antibody combination assays (so-called fourth generation assays) should be given priority in areas with high HIV incidence (which often coincides with high prevalence).

Antiretroviral treatment outcomes (paper II)

Survival was 96.3% at 12 months and 87% at 60 months after the beginning of ART. After 5 years of ART, the majority of children 710 (98.1%) were on first-line treatment, and 21 children (1.9%) were on second-line treatment, as shown in Table III. Since viral load testing was not regularly available, and switching to second-line treatment, as previously discussed, was essentially implemented when immunological failure (CD4 <15%) occurred, the number of children on second-line treatment was small. After 12 months on ART, there was an improvement in mean (SD) CD4% from 12.3% (6.3) to 27.7% (8.8) (p<0.001). There were also weight and height gains, as expressed by Weight-for-age Z score (WAZ) and Height-for-age Z score (HAZ). After 12 months of ART, the mean (SD) WAZ improved from -2.3 (2.2) to -1.1 (1.4) (p=0.05). The mean (SD) HAZ progressed from -2.7 (1.7) to -2.2 (1.4) (p = ns). Growth was improved, with emphasis on weight gain during the first year of ART, as shown by an improvement in
WAZ values. This growth improvement is in line with a good treatment response and is consistent across other paediatric studies. As shown with a Kenyan [210] cohort, a parameter such as weight can be a simple tool to assess treatment response in peripheral treatment sites in poor countries.

A cross-sectional assessment of viral load (VL) was performed in 2006 in 495 children on ART for more than 6 months; 360 (72.7%) had a VL of < 50 copies/mL of HIV-1 RNA. Tolerance to drug regimens was generally good, with only 28 of 1125 children showing severe toxicities. The major serious adverse events that necessitated a change in HAART were Stevens-Johnson syndrome, caused by nevirapine (n=8), clinically evident lipoatrophy syndrome, probably caused by stavudine (n=3), peripheral neuropathies, caused by stavudine (n=4), severe anaemia, caused by zidovudine (n=5), acute hepatitis, caused by nevirapine (n=3), nevirapine-induced rashes (n=5) and efavirenz-induced behaviour disruption (n=1).

In this cohort of previously antiretroviral-naïve HIV-1-infected children with moderate-to-severe disease and severe immunossupression, good clinical and immunological ART responses were achieved.

Survival after 60 months of ART was 87% and those who survived continued follow-up. Survival in our cohort is better than reported in similar studies. We have reported results after 60 months of ART whereas the available publications mention results in shorter periods. In Côte d'Ivoire [359], after 42 months of ART, survival probability was 0.86. Médecins sans frontières (MSF) [360], in a study conducted in 14 countries, showed a survival probability of 0.89 at 12 months of ART. In Haiti [208], the survival was 81% after 3 years of ART. In Cambodia [361], survival was 92% at 12 months and 91% at 24 months.

Viral load was cross-sectional, assessed in children who were given ART for at least 6 months, and showed that 72.7% had less than 50 copies/mL, which is comparable to what has been found in other settings. In similar studies, the percentage of children with undetectable viral load was 46.3% at 36 months and 45% at 42 months in Côte d'Ivoire [359], 39% at 9 months in Kenya [210], 81% in Cambodia [361], 49% at 24 months in South Africa [362] and 77.5% in Italy [363] in children at 5 years after early HAART.
Immunological response was good, with an improvement of CD4 percentage from 12.3% to 27.7% at 12 months. Similar immunological benefits were presented in other contexts. For instance, results from Côte d’Ivoire [359] showed a median CD4 percentage of 23.1% at 36 months. MSF [360] has shown in 14 countries that after 4 years of ART, 62% of children had CD4 percentages greater than 25%. In Zambia [215], there was an improvement from 12.9% at baseline to 28.4% at 24 months. In South Africa [362], there was an improvement from 13% to 25% at 12 months. Finally, in Italy, after 5 years of HAART, 97.5% of children treated had CD4 percentages greater than 25%.

Tuberculosis (TB) occurrence seems high in this cohort, with 246/1125 (21.9%), but in Mozambique TB is the most frequently diagnosed mycobacterium disease, with a prevalence of 250/100,000 inhabitants and a mortality rate of 124/100,000 in adults[364]. Data concerning children is not available, but we believe that HIV-infected children in Mozambique are highly affected by tuberculosis.

Tolerance was good, with less than 3% of children experiencing side effects that necessitated drug withdrawal.

Adherence was good, though its measurement relied on patient reports and drug pick-ups at the pharmacy. There was no specific instrument to precisely measure adherence.

Resistance to paediatric antiretroviral drugs (paper III)

**HIV-1 genotypic resistance** - Wild-type viruses were detected in only seven cases (7/84, 8.3%). No specific characteristics were found for this subgroup of children, especially concerning the level of viral load at the time of analysis (median: 81,378 copies/mL (IQ: 1716-470,543) in children with wild-type viruses vs. 20,830 copies/mL (IQ: 5396-100,266) in children with resistant viruses; p=0.12). The overall frequency of viruses showing genotypic resistance to at least one antiretroviral drug was 92% (77/84). The percentage of children infected with a virus with ≥1 major mutation conferring drug resistance to NRTIs was 88% and to NNRTIs was 92%. As expected, most children (77/84,92%) with virological failure had viruses carrying at least one mutation conferring complete resistance to first-generation NNRTIs (Table 1); the most frequently observed mutations were Y181C/I (n=27) and G190A/S (n=26), followed by K103N (n=23), K101E (n=15), V106A/M (n=10), Y188L/C (n=8) and M230L
Unexpectedly, five children (6%) had a virus with a combination of mutations conferring resistance to one of the new generation of NNRTIs, etravirine (Table 2). The different patterns conferring resistance to etravirine were as follows: K101E, V179A, Y181C, G190A (n=1), K101E, V179I, Y181C, G190A (n=1) and K101E, V108I, V179I, Y181C, G190A (n=1).

M184V, conferring resistance to lamivudine, was the most frequently observed mutation for NRTI resistance, being found in 88% (74/84) of children with virological failure (Table 3). Thymidine analogue mutations (TAMs), conferring resistance to zidovudine or stavudine, were observed in 15% (13/84) of patients, and 8% of patients had ≥3 TAMs. K65R was detected in one case and was associated with Q151M and M184V. Among the 77/84 children with at least 1 mutation, 3 had a virus resistant to one drug, 58 harboured mutated strains resistant to 2 drugs of their treatment regimen, and 16 harboured viruses resistant to all 3 drugs of their treatment regimen. The number of patients with viruses resistant to NRTIs to which they had not previously been exposed (due to cross-resistance) was n=5 (6%) for tenofovir, n=4 (5%) for abacavir and n=3 (3.5%) for didanosine. The NRTI multidrug resistance mutation Q151M, conferring resistance to all NRTIs, was found in 3 children (3.6%). Twenty children (24%) harboured virus with an extended spectrum of resistance, defined as resistance to the 3 drugs of the combination received by the child and/or to at least one of the following drugs to which the child had never been exposed: abacavir, tenofovir, didanosine and etravirine.

Viral subtype: A phylogenetic tree analysis of the sequences of the RT genes of HIV-1 strains from 84 children infected in Mozambique revealed a high degree of genetic homogeneity. Almost all HIV-1 strains were of subtype C (79/84, 94%). Two subtype A (2.4%) and 3 subtype D (3.6%) isolates were also found.

Risk factors associated with extended-spectrum resistance: Extended-spectrum resistance was found in 20 out of 84 viruses. Multivariate analysis of clinical and biological characteristics measured at baseline or at the time of virological failure identified treatment duration as being significantly associated with the risk of multi-resistance: 12 of 26 (46%) resistant children treated for more than 24 months but only 4 of 23 (17%) resistant children treated for 6-12 months displayed an extended
resistance profile (aOR: 6.67 [95% CI 1.24 to 35.93], p=0.015). The increase per month of treatment, after the first six months, was 1.09 (1.02 to 1.16; p = 0.007; Table 3).

The minority of children in whom viral replication was not controlled were at high risk of developing resistance, including an extended profile of cross-resistance in some cases. Almost all children with virological failure harboured virus resistant to lamivudine (88%) and/or nevirapine (92%), which were 2 antiretrovirals included in their treatment with low genetic barriers to resistance. The risk of developing resistance to stavudine or zidovudine was lower, occurring in only 15% of children. Although based on a relatively small number of genotypes, these data are very similar to what has been described in adult patients receiving similar antiretroviral combinations. An unexpected finding was the relatively high incidence of extended cross-resistance to drugs to which the children had not been exposed. For NNRTIs, the accumulation of several mutations under selective pressure from nevirapine can confer resistance to the second-generation molecule belonging to this class, etravirine. Resistance to etravirine was defined as the presence of ≥3 NNRTIs resistance mutations; by contrast, a single NNRTI resistance mutation confers resistance to nevirapine [365]. Similarly, replication under selective pressure of zidovudine or stavudine leads to an accumulation of thymidine-associated mutations (TAMs). Because of cross-resistance between different NRTIs, resistance to abacavir, tenofovir and/or didanosine was also observed. The major problems for subsequent treatment were the occurrence of thymidine analogue mutations, K65R and Q151M, which are multidrug resistance (MDR) mutations. The occurrence and pattern of mutations may depend on the viral subtype. Nearly all viruses identified in this study were subtype C, in agreement with a recent study evaluating the distribution of viral subtypes in pregnant women in Mozambique [32]. There is evidence suggesting that the use of tenofovir can rapidly select for K65R in subtype C viruses in cell cultures [366]. We found only one patient who was not exposed to tenofovir harbouring the K65R mutation; this mutation has been detected at the same or higher frequency in adults after failure of a first similar antiretroviral treatment in South Africa and Malawi [367, 368]. It is likely that K65R emerged after failure of a first-line regimen containing stavudine [367, 369, 370]. Conversely, the emergence of K65R was inversely associated with the use of zidovudine [367, 369], used in a third of the children in this study. The presence of TAMs (15% in our study) antagonises the selection of K65R.
The relatively higher frequency of V106M (6/10) vs. V106A (4/10) in the reverse transcriptase gene confirms previous reports that V106M is the more common NNRTI-resistance mutation in HIV-1 subtype C [348, 371, 372]. It would be valuable to assess the effects of viral subtype on the selection of extensive resistance to NNRTIs, including resistance to the new NNRTI etravirine.

Not surprisingly, we found a link between treatment duration and the emergence of these extended-spectrum resistance profiles. The main approach to preventing extended-spectrum resistance is to switch from a failing regimen as soon as possible, even if the replication rate is low. This strategy avoids the accumulation of TAM and NNRTI mutations, which compromise second-line treatment as it progressively becomes available in low-resource settings.

The proportion of children in this study for whom the efficacy of currently available second-line drugs was potentially impaired was of the same magnitude as that estimated elsewhere for adults. In Thailand, for example, 8% of adult patients with virological failure under first-line treatment had limited antiretroviral options for second-line treatment [373]. The virologic and clinical consequences of this extended-spectrum resistance profile on second-line treatment involving boosted protease inhibitors remain to be determined in paediatric settings. The deleterious consequence of delaying an antiretroviral therapy switch was recently demonstrated for adult patients receiving NNRTI-based combinations [374]. Drugs with low genetic barriers to resistance and/or cross-resistance-inducing effects, such as lamivudine and nevirapine, currently recommended by the WHO in first-line treatments, increase this risk. Depending on their availability and provided that they are well tolerated, drugs chosen for future therapies should be those with strong genetic barriers to resistance and the lowest possible potential to select for cross-resistance mutations.

Antiretroviral and chemotherapy outcomes in AIDS-associated Kaposi’s sarcoma (paper IV)

Thirty-two patients with biopsy-proven AKS presented at the hospital during the period from December 2003 to December 2008, representing 0.8% of the total HIV-infected children who attended during that period. Four presented at a very late stage of the disease and died in the first days of admission, before any treatment could be administered. The present analysis is based on the remaining 28 children. AKS was the
clinical presenting form and led to the diagnosis of AIDS for all of these children. None were diagnosed among the children already receiving HAART during the study period. Twenty (71%) were male and eight were female. The mean patient age was 8.3 (range 2-16) years, and the mean (SD) follow-up time was 27.3 (14.9) months. All were HAART naive. The type of KS was lymph glandular for 5, coetaneous for 10 and mixed for 13 children. One child had pulmonary involvement proven by bronchial biopsy, but limited investigation procedures available on site precluded a detailed evaluation of possible visceral involvement, notably in the digestive tract. With regard to TIS staging, 60.7% had T0 disease, 14.3% had T0 disease, and 53.7% had S0, meaning that this cohort predominantly included moderately ill, severely immunosuppressed children. The mean CD4 absolute count (SD) at presentation and before chemotherapy was 668 (1012), and the CD4 % was 16% (9.2%). Paclitaxel was given to all children in a median (IQR) delay after initiation of HAART of 1 (1-3) month. Twenty-one children received the complete 6 cycles as scheduled. Seven children received a shortened program (1, 2, 3, 4 and 5 cycles, respectively) because of early death (n=4), toxicity (n= 2) or transferred out (n=1). The median follow-up time of these children was 27 months (IQR 18-36).

Feasibility and tolerance of paclitaxel chemotherapy
Paclitaxel treatment was generally well tolerated. No allergic or anaphylactic reactions were noticed for any children in a total of 150 intravenous chemotherapy infusions. Overall clinical toxicity grades 1 and 2 included fatigue (n=5) and myalgia (n=3). Grade 3 and 4 adverse events involved biological disturbances (anaemia n=2, neutropenia n= 2, thrombocytopenia n=1), one case of unexplained massive oedema with hypoalbuminemia and rapid death, which was observed after the second infusion in one child. Undiagnosed disseminated KS in the digestive tract with intestinal albumin loss could be an explanation, but an adverse event related to paclitaxel, although never previously described, cannot be firmly ruled out. One child had transient cardiac arrhythmia that was only diagnosed by ECG after the second infusion; treatment was delayed for one week, but he is doing well (Table 3). During the chemotherapy cycle, the CD4 % progressed from a mean (SD) of 16% (9.2) to 29.2% (27.1) (p=0.02).
Response to treatment

No child experienced any improvement of coetaneous lesions during HAART and before chemotherapy. No child experienced immune reconstitution syndrome (IRIS). Chemotherapy was thus proposed for all children together with HAART.

Among 28 children treated, four died early during the chemotherapy period, one after the first infusion, 2 after the second infusion and 1 after the third infusion. The causes of death were infectious for 3 of the children (one sepsis and two pneumonias) and probably related to KS extension for one child (severe hypoalbuminemia). In the remaining 24 children, 5 had total and complete remission of lesions at the end of chemotherapy, and 19 had 60-90% improvement of coetaneous lesions. However, the progressive disappearance of KS lesions was continuously noticed in all children after chemotherapy cessation, leading to total remission of the lesions in a period of 1-10 months after the end of chemotherapy. Three children had recidivated the coetaneous lesions in the three months following the end of chemotherapy. In two children, however, the relapses spontaneously cured under unchanged HAART and without need of further chemotherapy. In one child, progressive extension of lesions and lymph nodes justified 5 additional paclitaxel injections without any significant effect. Finally, four children in complete remission died 10 months (n=1), one year (n=2) and two and half years (n=1) after the end of chemotherapy. These deaths were suspected to be non-chemotherapy related; 2 were caused by severe malaria, and 2 occurred in the community, the causes remaining unknown.

When we compared the children who were alive with those who died (all causes), there was no significant difference in age, CD4 % at baseline, CD4 % after chemotherapy or TIS staging. When we compared children with and without relapse, there was no significant difference in age, CD4 % at baseline, CD4 % after chemotherapy or TIS staging.

To our knowledge, this is the first description concerning AKS treatment in children. The therapeutic strategy, HAART followed by chemotherapy, follows adult recommendations. More than 70% of the children (60% with advanced disease) are in total remission from AKS at a median of 2 years after treatment. These results are encouraging, as morbidity and mortality of AKS is high in low-resource settings. This
observational study has some limitations. It does not allow specification of the individual roles of HAART and chemotherapy. We cannot exclude the possibility that some children with few lesions and good immune status could improve with only HAART, in the absence of chemotherapy. On the other hand, complete remission of severe AKS with combined HAART and paclitaxel supports this therapeutic strategy. In a study performed in South Africa, in which adult patients were randomized to receive HAART alone or HAART combined with chemotherapy, the group that received the combined therapy had a better clinical outcome [375]. A recent report from the Chelsea and Westminster HIV cohort also points to the fact that patients treated with HAART alone required further therapy for Kaposi’s sarcoma [376].

The classic KS chemotherapy with adriamycin, vincristine and bleomycin requires some expertise in chemotherapy, which is limited in the majority of centres treating HIV-1-infected children in sub-Saharan Africa. New chemotherapy drugs, such as liposomal doxorubicin and taxol derivatives, have the advantages of high efficacy, easy administration and lowered toxicity. Paclitaxel is less costly than liposomal doxorubicin and has already been used in children with a number of cancers, with a tolerance profile similar to that seen in adults. The doses we have used here are extrapolated from adults with AKS. We have confirmed paclitaxel feasibility and tolerance in the context of a resource-limited setting. The association of HAART and paclitaxel does not seem to be more toxic; moreover, paclitaxel does not impede CD4 improvement in children undergoing HAART. None of our patients presented with IRIS, which has been described in adults [377, 378]. It is possible that early chemotherapy has prevented the occurrence of IRIS.

Finally, it is interesting to note that in these 5 years, children presenting with AKS were those not yet treated by HAART. In the 1125 children on HAART, none presented AKS, confirming that better AKS prophylaxis is HAART through immune reconstitution.
7 Conclusions

After more than two decades since the discovery of HIV, the disease has massively spread throughout the world. Initially confined to homosexual men and drug addicts, the pandemic expanded to include impoverished women and children, the two populations most severely affected by the HIV pandemic. Despite the advent of antiretroviral treatment that has saved lives and has controlled the epidemic in affluent countries, impoverished countries were unable to access those treatments until nearly a decade after their discovery. Also, there has been a delay in the arrival of paediatric formulations to the market for the treatment of children infected with HIV. Fortunately, the recent arrival of fixed-dose combination treatment tablets and paediatric fixed-dose combination treatment tablets has made the massive scale up of treatment a reality. Despite an increase in the availability of antiretroviral treatment, only 30% of those infected with the virus actually receive the life-saving treatment. This pandemic, unlike others, has unveiled the weaknesses of health systems in impoverished countries. On the other hand, a large amount of money and resources have been allocated to strengthen the deteriorated health systems of resource-constrained countries due to the pandemic. This improvement of healthcare infrastructure has extended to other disease control programs for diseases such as tuberculosis and malaria. However, many difficulties still have to be faced in order to control the pandemic. These obstacles include the scarcity of qualified human resources, the absence of good program management, the lack of coordination between different health programs and NGOs, and the inadequacy of public education. An important and sustained effort is needed to improve education as a means for disease prevention and to improve country’s development. Also, an assessment of the antiretroviral treatment at both a national and regional level, in urban and rural areas, and in both public and NGO supported sites would provide useful information about the effect of the massive scale up of these treatments with special emphasis on paediatric treatment.

Uncertainty still remains in regards to the future of patients treated with the antiretroviral treatment in resource-constrained settings. There are many questions that arise from our minds, yet so many questions remain unanswered in this fight for survival against HIV. What future treatment options will be available and what
resources will be there to cope with these needs? What quality of life will the children have whose lives we are saving today? Will a therapeutic vaccine be available in the future? What are the future psychosocial and economic consequences of the today’s pandemic, and what are we leaving for future generations?

Despite these remaining questions, in Mozambique, we have shown that effective treatment can be administered to HIV-1-infected children for at least five years at a large scale. This is an important first step that benefits many children living in an impoverished country.

This hard work must go on with the main hope that our children will continue to smile!
8 Acknowledgements

I would like to take this opportunity to express my sincere gratitude to everybody who has helped me to reach my goal. It has been a long way full of fun, but plenty of obstacles, an interesting learning and maturing process. Therefore, thank you ...

Stéphane Blanche, my co-supervisor, my mentor and my friend who has encouraged and helped me along the way to build the infrastructure for treatment and care of HIV positive children in my country, for sharing your great knowledge and guiding me through all the process of research.

Sören Andersson, my supervisor, for giving me the opportunity of accomplishing this thesis, for your generosity, for never saying no, even when you didn’t believe and for all your support.

Gunnel Biberfeld, my co-supervisor, for your precious advises.

Swedish International Development Aid Agency (Sida)/ SAREC for funding the research project that allowed the PhD conclusion.

All my co-authors and collaborators for the great contributions to this work.

The children and families that gladly participated in this work. Your strength and courage dealing with this disease have taught me a lot.

The best team someone could ever dream to work with - the paediatric day hospital staff: Eugénia, Luisa, Sócrates, Catarina, Ester, Francisca, Alima, Elisa, Luisa M, Graca, Lucia, Aida and Sitefane. Your are not just colleagues, but friends and almost family! Your dedication to work and to the children’s cause has always been inspiring.

Calu and Orvalho, your help has been invaluable in setting up such an enormous database and keeping it always clean and manageable. A true experts’ task.

My colleagues from the department of Paediatrics: Maria Helena, Orlanda, Ana Graça, Isabel, Clementina, Benedita, Miguel Samuel, Valéria, Nancy, Faizana, Paula, Bia, Yassmin and all the others, for being such a great team.

Ilesh, who smartly leads one of the best laboratories in Africa, where colleagues like Adolfo, Dulce, Nelson, Nadia and others give their contribution to our children’s health.
Colleagues in the HIV program at the Ministry of Health, leaded by Américo and Marlene, who have been working hard to organize the provision of treatment to HIV-infected people in our country. I admire your work.

My colleagues that are now leading the Faculty of Medicine, Mamudo and Natércia always very supportive through the whole process, with wishes for courage and hope.

My partners on this struggle to achieve the PhD thesis: Sibone, Josefa, Eulália, Carolina, Sofia, Belisário, Noémia and Ane, on the way to work on their thesis. I wish you lots of success.

Colleagues at the Swedish Institute for Infectious Disease Control- Lindvi, Kerstin, Maria, Lotta, Eva, Barbrö, Anita, Karina, for all your support.

Partners with whom along the way we have faced many barriers, surpassed many obstacles and shared successes in providing antiretroviral therapies to HIV-infected children in Mozambique: Leila, Josué, Lisa, Marc, Ferrucio, Bia, João, Maria, Ligia, Luisa, Ligia, Fernanda, Marc, Aly, Viviane and all the others.

Krok, my best bro, our early morning bike rides along the coast filled with lots of laughs and reflections about life have been deeply precious.

My friends: Beth, Lena, Leninha, Rolanda, Maya, Nirmala, Vanda, Tó, Alda, Anita, Amélia, Gina, Paulo, Nela, Sandra, Salim, Pascal, Sergio, Isabel, Xiluba, Camilo, João, for your always present and never ending friendship.

My in-law family, Collette, Paul, Eliane, Jean-Loup, Sébastien, Carole and Marjorie, for all your thoughtful support.

My father, Eugénio, my brothers Guilherme and Frederico, my niece Barbara, my uncle Fernando, my auntie Ana and all my family, for always be there, lovely and supportive.

And my son Pablo, the joy of my life, a bright young man; may your future be oriented by hard work and deep dedication to the causes in which you believe.

I acknowledge the contribution of many other persons that are not separately named but whose work and support remain in my heart.
9 References


90. Dean C.M., Winkler C, Huttley GA, Smith MW, Allikmets R, Goedert JI, Buchbinder SP, Vittinghoff E, Gomperts E, Donfield S, Vlahov D, Kaslow R,


235. children, W.g.o.a.t.m.m.o.H.-i., *Guidelines for the use of antiretroviral agents in Pediatric HIV infection.* 23 February 2009, National Institutes of Health, USA.


Working Group on antiretroviral therapy and medical management of HIV-infected children-. *Guidelines for the use of antiretroviral agents in Pediatric HIV infection*. 2009, National Institutes of Health, USA.


