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Effectiveness And Monitoring Of Antiretroviral Therapy For Hiv-Infected Children In Accra, Ghana

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Effectiveness and Monitoring of Antiretroviral Therapy for HIV-Infected Children in Accra, Ghana

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Abstract

Title: EFFECTIVENESS AND MONITORING OFANTIRETROVIRAL THERAPY FOR HIV-INFECTED CHILDREN IN ACCRA, GHANA

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Despite advances in HIV diagnosis and care, many challenges remain in resource-limited settings. As scale-up of antiretroviral therapy (ART) progresses in resource-limited settings, evaluation of available first-line regimens and monitoring techniques is needed to ensure the safety and success of such efforts. This study had two specific aims. First, the study aimed to evaluate the effectiveness of first-line ART regimens at a large HIV program in Ghana where participants are predominantly infected with non-type B HIV virus. Second, the study aimed to investigate the potential value of laboratory-based biomarkers, specifically absolute CD4 cell count, CD4 cell percentage and HIV RNA viral load in predicting failure on ART. This study enrolled a prospective cohort of 87 HIV-infected children receiving care at Korle Bu Teaching Hospital in Accra, Ghana. Blood samples were collected a 4-6 month intervals and participants were followed until treatment failure, defined by WHO criteria, as the primary study outcome. 83% of participants demonstrated effective treatment response to first-line regimens with 0% associated mortality and only 4.7% switched regimens over the study period. Predictors of treatment failure included a shorter time interval between HIV diagnosis and ART initiation ($p=0.014$) and parents who are both HIV-positive ($p=0.05$). Lower mean baseline absolute CD4 cell count appears to be a significantly predictor for immunological failure ($p=0.041$). Absolute CD4 cell count and CD4 cell percentage after ART initiation showed similar trajectories and patterns. The findings demonstrated that available first-line ART regimens were effective, well tolerated and potentially durable in this setting with little evidence of resistance or toxicity. In settings in which CD4 cell percentage is not readily available, absolute CD4 cell count may provide an alternative biomarker for monitoring treatment response. These conclusions hold valuable public health implications in the scale-up of ART access and monitoring.

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1. INTRODUCTION

A. Advances in HIV management

The development, advancement and utilization of antiretroviral therapy (ART) has significantly affected the management and outcomes for all people infected with HIV. The use of ART, particularly combination therapy known as highly active antiretroviral therapy (HAART), has resulted in tremendous reductions in both morbidity and mortality for HIV-infected individuals, particularly those living in resource-rich countries. The rate of perinatal HIV transmission in resource-rich countries has been reduced by 98-99% following the advent of ART[3]. In the United States, there were only 67 children in all of 2007 that were born with HIV[4]. Remarkably, the last child born with HIV at Yale-New Haven Hospital was delivered in November 1996[5] and the hospital has just celebrated this 15 year record of achievement.

Beyond prevention of transmission, the use of ART has improved both the quality and the quantity of life for people infected with HIV throughout the entire world[6]. A particularly notable achievement in the pediatric population has been the positive effect that ART has brought to nutritional status. In the first year of ART, the average weight

gain for children is as much as 3.6 kg, which raises the mean weight-for-age *z* score by one standard deviation[3].

Due to the remarkable advances delivered through the use of ART, a worldwide effort has been undertaken to create universal access to ART. The scale-up of ART access in resource-limited settings is increasingly feasible due to a boost in global funding for HIV/AIDS in the previous decade combined with reduction in drug costs. In these settings, there has already been a remarkable increase in access. By the end of 2007, an estimated three million people were receiving ART in low and middle-income countries[7, 8]. This represents over a seven fold increase in the amount of people receiving ART in the previous four years[8]. The increase is likely due in part to innovative models of delivery, including home-based care delivered by trained local health personnel, which has demonstrated good health outcomes in rural Uganda[9].

The scale-up not only appears feasible, but it has been shown to be highly cost effective, even in the Africa-specific context[6]. A study done by Cleary et al. in Khayelitsha township near Cape Town, South Africa, found that ART is cost-effective at a cost of $1,300 per quality-adjusted life year (QALY) gained[10]. This study was conducted using data from a clinic providing comprehensive HIV care with a cohort of 1,729 adult patients followed for a minimum of four years on ART[10]. In Cote d'Ivoire, Goldie et al. also found that ART was highly cost effective, even in the absence of lab monitoring techniques, and demonstrated that ART was most cost effective when used in combination with antibacterial prophylaxis[11].

The health outcomes for patients receiving ART in resource-limited settings, Africa in particular, have been encouraging. In the pediatric population overall, ART in

resource-limited countries has been as effective as ART in resource-rich countries[3, 12]. Pediatric specific studies of the effectiveness of ART have been done in South Africa[13], Cote d'Ivoire[12], Kenya[14], Uganda and Zimbabwe[15]. At six months, survival on ART was 92% or higher[12] and survival at five years on ART was 87% or greater[15]. A large study done by Davies et al. in South Africa included outcomes for 20% of all children on ART in South Africa and demonstrated that mortality at three years on ART was as low as 7.7%[13]. Of particular relevance to our study, a database of pediatric HIV patients in West Africa from 2000 through 2008 was analyzed and showed 92% survival after twelve months of ART[16]. This study included nine clinical centers in six West Africa countries, including Ghana.

Furthermore, the children receiving ART in resource-limited settings have demonstrated low toxicity and similar issues of adherence and retention as in resource-rich settings. For children receiving ART in multiple centers run by Medecins Sans Frontieres (MSF) throughout Africa and Asia, only 3.8% of patients experienced severe toxicities[17]. In a study done in Kenya by Wamalwa et al., 10% of pediatric patients experienced severe adverse effects necessitating a switch in antiretroviral regimen[14], in a setting in which the majority of patients started on a regimen including two nucleoside reverse transcriptase inhibitors (NRTI) and one non-nucleoside reverse transcriptase inhibitor (NNRTI), which is similar to the other African-based pediatric studies. Adherence to ART in these studies appears good and similar, if not better than, studies in resource-rich countries. In Uganda, over 97% of patients were found to be taking at least 95% of their pills in any calendar quarter[9]. Retention of patients in ART programs

ranged from 73% to 81%, which is similar to studies in adults and in resource-rich settings[13, 16, 17].

B. HIV in Ghana

Ghana, as seen in Figure 1, is located on the West African coast, bordered by Togo to the east, Burkina Faso to the north and northwest, and Cote d'Ivoire to the west. The population is estimated at 24.5 million. Life expectancy at birth is 55 years for males and 60 years for females. The maternal mortality ratio is 451 per 100,000 live births. The infant mortality rate is 50 per 1,000 live births and the under-five mortality rate is 80 per 1,000 live births[1, 2].

Figure 1. Map of Ghana and administrative regions

The first reported case of HIV in Ghana was in 1986 and a national response to the disease was organized in 1987. In 2000, the Ghana AIDS Commission was established to coordinate the management of HIV/AIDS programs nationwide. The national prevalence of HIV is estimated to be 1.9% in 2009, a decrease from 3.6% in 2003[2, 18]. The highest prevalence is among pregnant women, ages 30-34, where 2% to 4.2% are infected, which illustrates the critical importance of prevention of mother-to-

child HIV transmission (PMTCT) programs[2]. There is a marked variation of HIV in pregnancy by region, as shown in Figure 2, with higher prevalence rates in regions with larger urban populations.

In Ghana, HIV-1 viral infection is predominant, accounting for 94.4% of infections and dual infection with HIV-1 and HIV-2 account for 4.4%[19]. HIV subtype A and circulating recombinant form (CRF) A/G are dominant in Ghana[19].

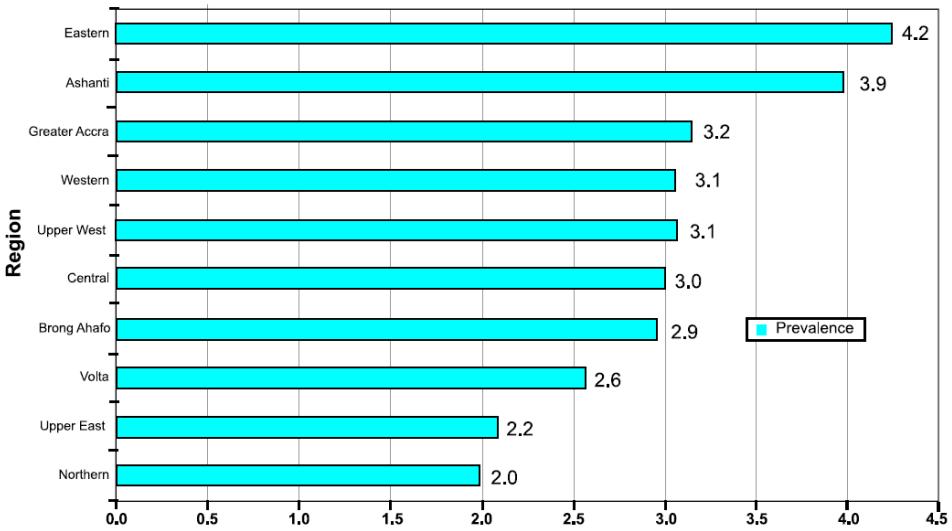

Figure 2. HIV prevalence rates in Ghana by region (*Source: National Guidelines for Prevention of Mother to Child Transmission of HIV*[2])

Approximately 267,000 people are living with HIV in Ghana and approximately 9% of those infected are below the age of 15 years[2]. In 2009 alone, 2,566 children died from HIV/AIDS in Ghana[2]. The social effects of HIV on children and families are far reaching. The Joint UN Programme on HIV/AIDS has estimated that as many as 250,000 children in Ghana have lost at least one parent to HIV[18].

As stated above, one area of critical importance in Ghana is PMTCT. In 2009, 3,354 babies were born HIV positive in Ghana and mother-to-child transmission accounted for 15% of all new cases[2]. As shown in Figure 3, there are many barriers in

the PMTCT cascade of management. The first barrier is registration for antenatal services and PMTCT as needed. Only 53% of all pregnant women even accessed antenatal services with HIV testing and treatment available. Testing is the second barrier in the PMTCT cascade for women in Ghana, where only 74% of pregnant women registered in PMTCT programs were tested for HIV in 2009, which represents only 39% of all pregnant women in Ghana[1]. This barrier limits the access of infants to early diagnosis and prophylaxis treatment. Only 30% of HIV-exposed babies in Ghana received ART in 2009[1].

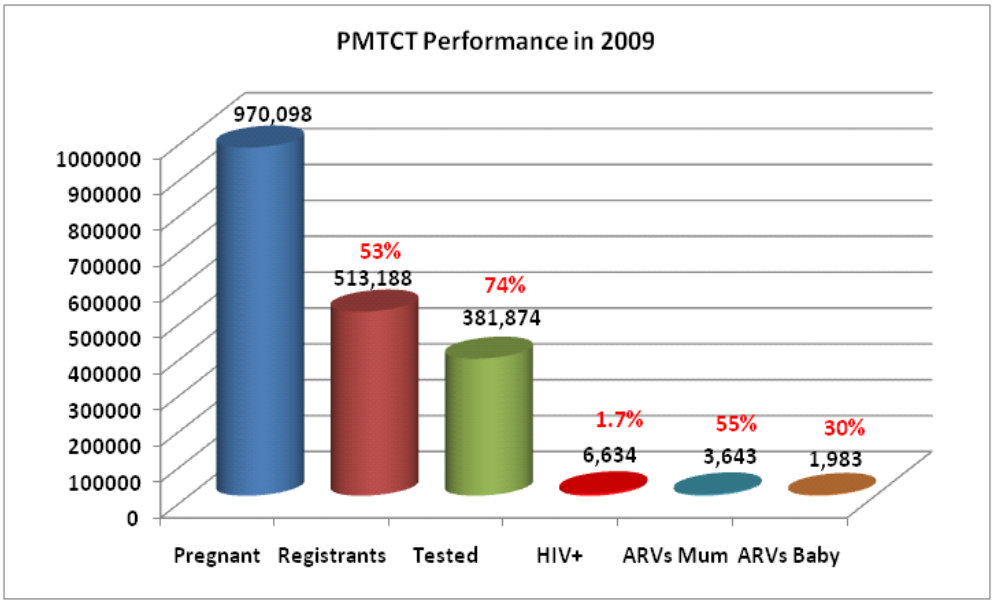

Figure 3. PMTCT statistics in Ghana in 2009 (*Source: Prevention of Mother-to-Child Transmission of HIV in Ghana*[1])

Despite national and global scale-up of ART, access in Ghana is still a significant concern. In 2009, approximately 71,000 Ghanaians were in need of ART, but only 14% of them received it[2]. Only 7% of those receiving ART were children – a disproportionately low rate of access given that children represent 9% of all HIV positive Ghanaians[2]. One of the factors potentially limiting access to ART is continued

stigmatization of HIV within communities. In Ghana, 74% of women and 64% of men would not buy fresh vegetables from a vender with HIV[18]. Only 21% of children younger than 15 years have been informed about their disease by their parent or guardian, which illustrates the lack of communication and persistent stigma surrounding HIV in Ghana[20].

C. On-going HIV epidemic

Despite advances in treatment and access to testing and ART, mother-to-child HIV transmission continues to raise the pediatric incidence of HIV in developing countries. Worldwide, there are 1,200 new HIV infections in children less than 15 years old every day[21]. 90% of these pediatric infections occur in developing countries and more than 90% are due to mother-to-child transmission[21]. Although dramatic increases in the number of patients receiving ART have been documented, the majority of children in need are still not receiving therapy. As stated above, in Ghana, only 14% of those in need received ART in 2009[2]. Globally, children represent only 6.6% of the three million people receiving ART[17]. Achieving universal coverage will be a major public health challenge, particularly in sub-Saharan African countries with high prevalence. For example, in South Africa alone, it is estimated that more than six million people will require ART in the next ten years[10].

One major factor affecting both prevention of HIV transmission, in the antenatal population, and mortality, in the pediatric population, is early diagnosis. Only 15% of HIV-exposed infants receive a diagnostic test within the first two months of life[21]. This

lack of early diagnosis translates to significant consequences in child mortality. One-third of infants die before 1 year, half will die before 2 years and three-quarters of children succumb before age 5 without treatment[17, 21]. Subsequently, the age at ART initiation in resource-limited settings is high, underlining the limited access and coverage of testing and treatment services. In a large West African cohort, the mean age at ART initiation was 4.9 years[21] and in Ghana, the median age is 5.5 years[22]. Delayed ART initiation can be associated with advanced clinical stage and level of immune suppression at initiation[22] and has effects both on the short-term and long-term health outcomes. In children of all ages, it has been demonstrated that lower CD4 counts at initiation are associated with impaired recovery and T-cell reconstitution[23], which prolongs the period of vulnerability to opportunistic infections[24]. In the long-term, children who initiate ART at an older age had lower age-adjusted CD4 counts after a five year follow-up period, which indicates that the younger, immature immune system's ability to recover from HIV infection may decrease with age and duration of infection, further emphasizing the critical need for early diagnosis and treatment[23].

Many challenges and barriers to universal access and care remain in resource-limited settings. First, inefficient or non-existent healthcare infrastructure challenges access and delivery of care. Lack of record keeping and medical documentation is a infrastructure issue noted frequently in resource-limited settings and is a major barrier[3]. At a large treatment center in Cote d'Ivoire, patients experienced involuntary ART interruptions because of stock exhaustion of ART drugs at the central administrative level, exemplifying the challenges of infrastructure in HIV care[12].

A second barrier is the limited range and availability of ART formulations. As in the Cote d'Ivoire example, ART may not always be available but even when available the different formulations may be sub-optimal. First-line regimens in resource-rich countries are protease inhibitor (PI)-based therapies[25], as a consequence of research demonstrating reduction in overall mortality versus other combinations[14, 26]. However, in resource-limited settings, NNRTI-based formulations remain the most common due to the low availability and high cost of PI-based regimens.

Third, there are limited numbers of trained healthcare professionals, particularly physicians. The lack of trained providers, in large part due to "brain drain", compounds the issue of inefficient healthcare infrastructure and access to care. Finally, there is only limited funding available to support activities, particularly in settings with many competing public health priorities[10]. This can lead to a challenging ethical and practical situation where funds could be used either to treat fewer patients more comprehensively or to use the funds to provide more patients with fewer services[10]. Furthermore, significant and associated health issues persist even with access to ART. Pre-existing malnutrition or immune suppression still leads to significant morbidity and mortality in the short- and long-term[27]. Immune reconstitution inflammatory syndrome is a significant cause of mortality in the first three to six months of ART, and resistance and non-compliance continue to be major long-term issues[27].

D. Monitoring HIV in children

As the scale-up of ART advances in the developing world, one area that needs to be addressed is the monitoring of treatment and disease progression. ART is currently a life-long treatment with potentially toxic adverse effects and vulnerability to the development of virus resistance. However, current antiretroviral regimens have led to dramatic reductions in toxicity due to the development of new drug classes and in viral resistance due to the use of combination therapies. ART remains a long-term therapy and therefore a feasible and cost-effective monitoring strategy for resource-limited settings is an important and high priority, and a topic receiving an increasing focus[7]. If context-appropriate strategies and technologies are not developed, the success of ART scale-up could be jeopardized because of the critical current shortage of laboratories with the technical ability to perform monitoring tests, such as CD4 count and viral load[28].

In Africa, ART is often monitored without any routine laboratory monitoring, which is limited by healthcare infrastructure and cost. The state of healthcare infrastructures and the current costs of laboratory techniques has led some researchers to theorize that universal access to ART can only be achieved if done without routine monitoring[29]. However, research has demonstrated both the added benefit of monitoring on patient outcomes as well as the cost-effectiveness of lab-based monitoring techniques, particularly CD4 monitoring. The DART Trial in Uganda and Zimbabwe showed that routine CD4 monitoring is superior to clinically driven monitoring with a significant decrease in disease progression and mortality for patients receiving lab testing on a routine basis[15]. This study further found that routine CD4 cell count monitoring lead to significant decreases in disease progression and mortality compared to a group receiving ART without routine monitoring[15].

Currently, there are dichotomous standards of pediatric HIV care based on the patient care setting. For patients in developed, resource-rich settings, ART is initiated at higher CD4 counts and routine, frequent monitoring is undertaken. The U.S. Department of Health and Human Services advises the initiation of ART at CD4 cell counts less than 500 or for patients with symptomatic disease and CD4 cell counts above 500[30]. Monitoring, including CD4 cell count, CD4 cell percentage and HIV viral load, is recommended every three months[30]. In the U.K., similar resource-intense regimens are recommended including the use of viral load testing at ART initiation, 2-8 weeks after initiation and then every 3-4 months thereafter[7]. In fact, these resource-intense monitoring strategies have been found to be relatively cost-effective at a cost of $3,956 per QALY gained[30]. However, at the described level of cost, these strategies would no longer be cost-effective in a low-income country.

The monitoring of ART in resource-limited settings is a distinctively different strategy. As set forth by the World Health Organization (WHO), ART is initiated for children less than five years old at a CD4 cell percentage less than 25%, and a CD4 cell count less than 350 for children five years and older[31]. CD4 monitoring is recommended every six months and viral load testing is not part of the WHO guidelines[31]. Increased frequency of CD4 testing, the use of viral load and resistance testing, all represent additional system costs that may not be realistic with limited resources, current technologies and lack of healthcare infrastructure[3]. The difference in strategies and recommendations illustrates the need for research focus on this topic, particularly in light of the demonstrated benefits with routine laboratory-based monitoring. As more children

are treated with ART, the monitoring strategy and practice must be evaluated and optimized for best patient outcomes.

The most important parameter for monitoring HIV progression and ART has been the CD4+ T lymphocyte cell count. The use of CD4 cell counts provides a quantitative guideline for treatment initiation prior to the development of clinical symptoms, which has been proven to reduce morbidity and mortality[32]. As a screening tool for ART initiation, CD4 cell counts are significantly more specific than clinical criteria, which would fail to identify up to 70% of eligible patients without CD4 testing[6]. Studies in South Africa and Cote d'Ivoire strongly support the use of CD4 cell counts as a relatively low-cost technique to guide ART initiation in ART scale-up programs[6].

As a monitoring tool for ART and HIV disease progression, CD4 cell counts have enhanced the benefits of ART and have done so in a cost-effective way. In a study done in Cote d'Ivoire, the use of routine CD4 cell count monitoring increased life expectancy for patients at least one full year[11]. Kahn et al. found that in Uganda the use of CD4 cell count monitoring is even more cost-effective than ART alone, and particularly more cost effective than the addition of viral load testing[9]. The WHO, in its guidelines, recognizes the value and the limited access to CD4 testing, recommending that in areas with limited access to CD4 testing, the use of these resources should be targeted based on clinical events[21].

In the management of pediatric HIV, monitoring CD4+ T-lymphocyte counts is done using both CD4 cell count and CD4 cell percentage. In all children, CD4+ T-lymphocyte counts are very high during early childhood and physiologically decline as the child ages and immune system develops, stabilizing at similar levels as adults after

five years of age[33, 34]. Furthermore, wide variations in CD4 cell count between and within individuals has been demonstrated[35]. Therefore, due to the variation of CD4 cell count between both individuals and age groups, CD4 cell percentage has been used as a potentially optimal biomarker. The CD4 cell percentage may remain stable across age groups and individuals and could be used as a parameter for the initiation of ART and monitoring of treatment in children[24].

While CD4 cell percentages were previously the recommended parameters for all pediatric age groups, the guidelines are now shifting both in the U.S. and internationally, towards increased use of absolute CD4 cell counts. Research has shown that CD4 cell counts normalize to adult values at approximately six years old and the CD4 cell count thresholds used in adults have similar prognostic value in children and adolescents[36-38]. The WHO now recommends the use of absolute CD4 cell counts for all children from five years and older[31].

Additionally, there is growing research in the U.S. and Europe that suggests absolute CD4 cell counts could be reliable alternatives to CD4 cell percentage. There can be significant discordance between CD4 cell count and cell percentage when used as a screening tool for pediatric ART initiation. As many as 50% of all pediatric patients eligible for ART by at least one CD4-based criteria (either absolute cell count or percentage) have discordant values[39]. However, statistical models suggest that the CD4 cell percentage had no significant prognostic value over and above that of absolute CD4 cell count, irrespective of age[39]. In a New Haven pediatric HIV cohort, Paintsil et al. recently demonstrated that the absolute CD4 cell count is as reliable as using CD4 cell percentage in the monitoring of disease progression, regardless of the ART regimen[40].

This cohort included 97 participants and was followed over a ten-year period but had small numbers in the 0-5 year age group, limiting the power of the study to report on differences in younger age groups.

Viral load testing is another parameter used in the monitoring of HIV disease progression. Landesman and Burns postulated the value of viral load monitoring in 1996[41] and their ideas have been confirmed in subsequent research. First, viral load is perhaps the most valuable indicator of response to treatment[42]. CD4 cell counts shortly after ART initiation do not reflect the HIV viral burden and therefore viral load testing is a more sensitive biomarker to detect virological failure[7, 42]. Therefore, as an early indicator of treatment effectiveness, viral load testing can be used to guide changes in treatment regimen. WHO recommends the use of viral load testing prior to change in treatment regimen[31] and regimen switching based on viral load testing leads to earlier switching and increased life expectancy as compared to regimen switch based on CD4 counts alone[8, 30]. CD4 counts may take months to start declining to levels meeting immunological failure criteria after virological failure[3], which additionally increases the potential accumulation of resistance mutations[29]. The second demonstrated value of viral load testing is as an indicator of disease progression. Baseline viral load levels are independently associated with overall mortality[43] and changes in viral load are predictive of progression to AIDS[42] and other significant clinical events[27]. These findings have been confirmed in the African context by Obimbo et al in a study evaluating viral load burden and infant mortality[44].

Despite the certain value of viral load testing, there are limitations to its use, particularly in the resource-limited setting. First, children exhibit different viral load

responses to treatment in comparison to adults. Asymptomatic children are less likely to achieve viral suppression as compared to adults and may even have high viral loads that fluctuate or remain high in spite of strong adherence and CD4 count recovery[40]. Additionally, the decline in CD4 cell counts may not correlate with the viral load levels, a finding reported by Rodriguez et al[40]. Finally, the high cost of viral load testing makes its routine use in a resource-limited setting widely prohibitive. As a screening tool for ART initiation, viral load testing would cost $1700 per person identified in addition to the cost of CD4 testing[45]. As a tool for routine disease monitoring, viral load testing would have approximately ten times the cost per disability-adjusted life year (DALY) compared to schemes of clinical monitoring plus CD4 testing[9].

Due to the limitations of both CD4 testing and viral load testing, alternative biomarkers have been studied. In particular, total lymphocyte count (TLC) has been evaluated for use as an alternative to CD4 testing in resource-limited settings. TLC testing is low cost, utilizes an automated hematology analyzer and therefore requires minimal technical expertise, and has been proposed as an alternative to CD4 testing until those techniques are more widely available[46, 47]. In resource-limited settings of South Africa and in the U.S., TLC has been found to be positively correlated with absolute CD4 count and an independent predictor of mortality[48-50]. However, in a meta-analysis, there was a weak correlation between TLC and CD4 cell percentage[46]. Based on the demonstrated value of CD4 testing, both absolute cell count and cell percentage, as low-cost methods are further developed and widely accessed, alternative biomarkers such as TLC will not be necessary.

E. Technological advances

As access to ART scales up with the goal of universal access, low cost methods of monitoring treatment effectiveness and disease progression are a priority in resource-limited settings. Development of CD4 technologies is on the forefront of the advances. The major factors limiting widespread use of CD4 testing done by conventional flow cytometry are cost and technical expertise[6]. Flow cytometry techniques and instruments require technical expertise to prepare and process specimens, are associated with high cost of equipment and maintenance as well[28, 51].

New, non-flow cytometry based techniques, are rapidly being developed. Two promising technologies are Dynabeads® by Invitrogen, and the Daktari CD4 by Daktari Diagnostics, Inc. Dynabeads® is a cell separation technology using magnetized spherical beads[52]. This technology has been in use for various cancer research and is associated with a lower cost compared to current flow cytometry-based methods[51]. The feasibility, cost and accuracy of the Dynabeads® technology was evaluated in studies including five West African countries – Burkina Faso, Cote d'Ivoire, Senegal, Togo and Mali. This study by Diagbouga et al. found that the Dynabeads ® techniques were strongly correlated with the results of flow cytometry from the same samples and at a significantly lower cost than flow cytometry techniques[51]. The Dynabeads ® techniques were easily implemented at the six different research sites and cost $3 per assay, compared with the $25 per assay for flow cytometry[51].

The Daktari CD4 instrument is a portable, point-of-care CD4 assay that utilizes two non-flow cytometry techniques – microfluidic cell chromatography and lysate

impedance spectroscopy[53]. Microfluidic cell chromatography is a rapid cell separation technique shown to have high efficiency and purity[54]. Lysate impedance spectroscopy is a cell counting, non-optical, technique using measured changes in intra- and extracellular electrical conductivity[55]. This instrument is currently being further studied to evaluate accuracy and performance but would potentially provide a low-cost and highly mobile point-of-care system, adaptable and appropriate for far-reaching ART distribution programs.

Despite the advances and innovation, these new technologies, and others like them, do not have the capacity to compute CD4 cell percentage[40]. This limitation is especially relevant in the pediatric HIV population in which CD4 cell percentage is the recommended biomarker for monitoring for children less than five years old. Therefore, the pediatric HIV community currently, without further research in use of absolute CD4 cell count, is not poised to take advantage of these technologies when they do become widely available.

2. SPECIFIC HYPOTHESES AND AIMS

This study had two specific aims. The first specific aim (1) was to evaluate the effectiveness of first-line ART regimens at a large pediatric HIV program in Ghana. It was our hypothesis that first-line ART regimens at Korle Bu Teaching Hospital in Accra would be highly effective in this resource-limited setting of non-type B HIV-1 infection.

The second specific aim (2) was to evaluate the prognostic value in laboratory-based biomarkers, specifically absolute CD4 cell count, CD4 cell percentage and HIV RNA viral load in predicting treatment failure on ART. It was our hypothesis that absolute CD4 cell count can be used as an alternate and equivalent biomarker to CD4 cell percentage in a resource-limited setting and in children of all ages, for the monitoring and evaluation of treatment response and HIV disease progression.

The specific aims of the study have two significant public health implications. The first public health implication is regarding the choice and array of available antiretroviral treatments in Ghana. Currently, the selection of available antiretroviral regimens is based on historical research conducted in resource-rich settings with different HIV subtypes, and limited in Ghana by cost. By evaluating the current ART regimens in Ghana and their effectiveness, this research can offer critical comparative analyses on the existing regimens for naïve patients and make meaningful suggestions regarding the development of effective yet affordable regimens in order to avoid drug resistance. Resistance to currently available regimens for treatment-naïve patients would have potentially disastrous consequences as ART scale-up progresses. Additionally, based on

the effectiveness data, this research could suggest the vital need for greater production of the component parts of current regimens, promoting increased generic manufacture of current regimens and thereby boosting access to treatment.

The second public health implication of this project is to demonstrate the value of absolute CD4 count in a resource-limited setting in order to promote its acceptance and use in pediatric populations. Consequently, emerging point-of-care absolute CD4 technology could be made available for use and study in pediatric HIV populations. The use of these technologies would have the potential to increase access to ART initiation in rural, difficult to reach communities and enhance the ability to monitor ART in children, thereby reducing toxicity and drug resistance.

3. METHODS

This study was a two-year project with specific aims as listed in the previous section. The study was reviewed and approved by the Ethics and Protocol Review Committees of the University of Ghana Medical School and the Human Investigation Committee of Yale School of Medicine.

The study was conducted in three parts. The first part of the study (1) involved the enrollment of study participants, which included recruitment, consent and initial interviews. The second part (2) was the prospective collection of data at follow-up clinic visits. The third part of the study (3) was the statistical analysis of the data collected.

A. Enrollment

The study was conducted at the Korle Bu Teaching Hospital (KBTH) in Accra, Ghana, which is the largest hospital facility in Ghana and the third largest in all the West Africa region[56]. The hospital, as a whole, has approximately 2,000 inpatient beds, is the primary teaching hospital for University of Ghana Medical School, and is the primary referral center for at least the Greater Accra and Eastern regions of Ghana. However, patients in reality traveled to KBTH from all over the country and West Africa region.

The Department of Child Health at KBTH was the main site for this study. The Child Health Department at KBTH provides the highest level of pediatric care in the nation and has an impressive volume of cases, including the following annual statistics[56].

There are 11,000 inpatient admissions reported annually including 2,000 neonatal intensive care admissions. Additionally, there are 36,000 outpatients visits reported and 10,500 patients are being followed routinely at sub-specialty clinics at the Department of Child Health.

This study recruited and enrolled a prospective cohort comprised of HIV-infected children receiving care at KBTH. All children received care through the Pediatric HIV/AIDS Care program at KBTH. This program was established in 2004 and provides comprehensive HIV/AIDS care and management of opportunistic infections for HIV-infected children. The pediatric HIV clinic is operates on a weekly basis and patients are referred to this clinic through one of several channels. To be referred, children either: have mothers known to be HIV-positive during pregnancy and were participating in the program for prevention of mother-to-child transmission (PMTCT); are discovered to be infected after presenting with an AIDS-defining illness; or are diagnosed after a symptomatic sibling or parent was found to be HIV-positive. The majority of HIV-positive children are infected in the perinatal period and the Pediatric HIV/AIDS Care program now cares for over 1,100 children including those with a confirmed diagnosis as well as HIV-exposed infants who are pending final status determination.

Potential participants were identified through collaborative efforts between research assistants, physicians, pharmacists and counselors. The inclusion criteria were age between 0 and 12 years, confirmed HIV infection and naïve to ART. Children were defined as having a confirmed HIV infection if rapid serum antigen and antibody testing were positive after 18 months or they had two detectable HIV RNA levels using PCR after delivery as per WHO guidelines[57].

All participants received written and verbal information about the study and gave consent before enrollment along with assent from parents or guardians. These discussions took place in a private and confidential space at the Department of Child Health. A written consent form was developed in English. If the participant or guardian did not speak English, a translator was available and would assist in explaining the details of the consent. If participants or their guardians were unable to write, they provided their fingerprint with ink, as is a common practice for illiterate individuals in Ghana, in lieu of a written signature, to indicate their consent. All participants received a copy of the consent form after signing.

After giving written consent, participants were enrolled in this study and were given a unique number and no identifying information was kept with study data. Enrollment began in September 2009 and continued through May 2011. At the time of enrollment, participants and guardians were interviewed to document demographic and background information including HIV diagnosis date, mode of transmission (if known), ARV start date, ARV regimen, parental status (if known), parental HIV status (if known), participant's tuberculosis (TB) status and history, past PMTCT treatment for mother, status and HIV status of participant's siblings (if known) and the participant's disclosure status (whether the child knows his/her diagnosis). As at any time during the study, if the participant or guardian did not speak English, a translator was provided. Pediatric HIV/AIDS Care Program files as well as hospital files were reviewed at the enrollment interview. These medical records were reviewed to validate background information verbally reported to research assistants as well as to collect clinical data preceding

enrollment such as prior WHO staging, previous CD4+ T-lymphocyte testing, previous HIV viral load testing and growth data (weight and height).

B. Data collection

The second part of the study was the prospective data collection after enrollment and ART initiation. The first study visit and data collection was near the time of enrollment. Blood samples were obtained for CD4 absolute cell count and cell percentage, HIV viral load and complete blood count. The first data point was within three months, either before or after, ART initiation. If tests, such as CD4 testing, had already been performed at the time of enrollment and within three months of ART initiation, these tests were not repeated and the preceding data was collected.

After enrollment and the initial study visit, participants were seen and examined at the pediatric HIV clinic at least every 4-6 months and more frequently as necessary. At each visit, the participant's weight and height were measured and the patient's WHO staging was updated. Every 4-6 months during the study period, blood samples were collected and CD4 absolute cell count and cell percentage, HIV viral load, and complete blood count were tested. Additionally, participants and guardians were interviewed at each follow-up visit to assess adherence to therapy. Adherence was measured in several methods. First, participants and guardians were asked to self-report the number of doses missed by the participant in the previous three days. Second, dates and timing of pharmacy refills were cross-checked if adherence was questioned. Additionally, missed clinic appointments were also documented for use as a potential marker of participant

adherence. Medical records were also reviewed at follow-up visits to recognize and document interim hospitalizations and unscheduled clinic visits to identify opportunistic infections.

All blood samples were processed at laboratories at KBTH. CD4 absolute cell count and cell percentage were quantified by a dual-platform flow cytometry technology using a FACSCount system (Becton-Dickinson, Franklin Lakes, NJ, USA) at the clinical laboratory at the Fevers Unit at KBTH according to manufacturer's instructions. Samples were processed within four hours and results were delivered on the day following delivery. The laboratory is certified by the South African Public Health Reference Laboratory and participates in an external quality assurance testing program by the South African Public Health Reference Laboratory. The HIV RNA viral load testing was performed at the Virology Lab at KBTH. In this lab, the COBAS ® AMPLICOR Monitor test (Roche Diagnostic Systems, Branchburg, NJ, USA) was used for the quantification of the HIV-1 RNA with a detection limit of 400 copies/mL. The testing was performed according to the manufacturer's instructions. Starting in September 2011, some viral load testing was performed at the Central Laboratory at KBTH after viral load instrument were procured. At this laboratory, the COBAS ® TaqMan ® 48 Analyzer (Roche Diagnostic Systems, Indianapolis, IN, USA) was used with a detection limit of 20 copies/mL. When processing blood samples for all viral load testing, an additional plasma sample was set aside and put in frozen storage for potential future use in resistance testing and other research.

C. Statistical analysis

The statistical analysis for this study was undertaken in accordance to the specific aims of the study.

i. Effectiveness of first-line ART regimens

The primary outcome of the study was treatment failure, either virological or immunological failure. The criteria used for treatment failure was the criteria set forth by the WHO[31]:

- **Virological failure** was defined as a viral load > 5,000 copies/mL after at least 24 weeks on ART.
- **Immunological failure** was defined based on age. For children between 2 and 5 years, immunological failure was defined as absolute CD4+ T-lymphocyte count persistently < 200 cells/microliter or CD4+ T-lymphocyte percentage < 10 % after at least 24 weeks. For children 5 years and older, immunological failure was defined as absolute CD4+ T-lymphocyte count persistently < 100 cells/microliter after at least 24 weeks.

After identification of potential treatment failures, based on laboratory results using the criteria above, particularly those with viral load greater than 5,000 copies/ml after 24 weeks on ART, participants were seen in the clinic sooner than the standard 4-6 month follow-up period. The purpose of the clinic visit was for a clinical assessment and

full interview by the team of physicians to assess the level of medication compliance. The participant's medical records were scrutinized, particularly the pharmacy refill schedule, and the participant and guardian were counseled about the importance of adherence to the ART regimen. Repeat blood samples were then collected at the next clinic visit, which was scheduled 4-6 months after the previous sample collection. Based on the two follow-up visits and repeat laboratory measurements, the participants were determined to be either treatment failures or non-compliant. For participants failing treatment, physicians made subsequent decisions to change ART regimens. These participants had reached the primary outcome of this study and were no longer followed. For participants deemed non-compliance, further counseling was conducted and the participant remained on the first-line regimen. These participants continued to be followed in this study.

To determine associations between baseline characteristics, statistical analyses were performed using SAS (SAS Institute, Cary, NC, USA). Participants were excluded from analysis if they did not have a data point 24 weeks or later after ART initiation. For baseline demographic characteristics, Chi-square and Fisher's exact tests were used. For baseline laboratory variables, Wilcoxon Rank-sum tests were performed. To analyze agreement between immunological and virological failures, a 2x2 table was produced and the kappa statistic was calculated.

ii. Use of absolute CD4 cell count

To analyze individual short-term responses to ART based on absolute CD4 cell count, CD4 cell percentage and viral load, spaghetti plots were constructed using baseline data points and subsequent data points up to 30 weeks following ART initiation. To analyze the use of absolute CD4 cell count as an alternative to CD4 cell percentage, graphs depicting the trends and trajectories of absolute CD4 cell counts and CD4 cell percentages over a 24-month follow-up period were produced. These graphs were used to identify trends in the data and descriptively analyze any similarities or differences. Data points from participant laboratory values were grouped according to timing relative to ART initiation and designated to a clinic visit number representing 6-month intervals (i.e., visit 1 represents baseline, visit 2 represents 6 months after ART initiation, visit 3 represents 12 months, etc.). Data were designated to clinic visit number if the data was collected in a 3-month interval surrounding the visit number.

D. Author responsibilities

I was the second Doris Duke Clinical Research Fellow involved in this two-year project. As such, I was specifically and personally responsible for:

- **Adjustments and improvements to the study protocol**

I was personally responsible for the identification and definition of specific inclusion criteria for this project, in particular the timing between enrollment and initial data collection and the enrollment of only ART-naïve patients.

- **Enrollment of study participants in the second year of the study**

I identified, recruited and enrolled participants in this study from September 2010 through May 2011. During this time period, I enrolled 33 participants. I conducted the initial interviews at enrollment and with the assistance of Mr. Jonas Kusah, our research assistant and translator, as needed. I was also responsible for the review of the participant's clinic file, hospital file and all laboratory results at this initial visit.

- **Tracking of study visits and collection of study samples**

I developed computer-based systems for tracking the visits and sample collections of all enrolled participants so as to minimize lost-to-follow up and inappropriate time intervals between participant sample collections. I was responsible for the tracking of 91 enrolled participants and collection of study samples at appropriate time intervals. During my time in Ghana, I coordinated 174 study visits for enrolled participants in which study samples were collected. At all study visits, I conducted the interviews to monitor ART adherence, reviewed the patient's medical records to identify interim hospitalizations, opportunistic infections and missed appointments. I delivered blood samples to the various appropriate laboratories, collected the results and inputted all data into our project database.

Mr. Sampson Bonney at the Child Health Laboratory collected the blood samples. The CD4 testing was conducted in the Fevers Unit by lab technicians. Mr. Isaac Kofi Badu, Mrs. Makafui Seshie and Mr. Isaac Boamah conducted the viral load testing in the Virology Lab with supervision by Dr. Kwamena Sagoe.

Furthermore, while I was in Ghana, the physicians working in the HIV clinic expressed strong interest in the immediate clinical use of our longitudinal study data. Thereafter, I established a computer-based, secure and sustainable system for the study data to be available to all clinic physicians on a real-time basis thereby maximizing the clinical relevance of the research study data.

- **Preliminary data analysis**

I performed the preliminary data analysis near the end of my fellowship in Ghana. The analysis was an integral part of a presentation I made to the Department of Child Health staff in May 2011. This data analysis was also used in several abstract preparations and poster presentations at Yale Student Research Day and the Doris Duke Clinical Research Fellowship Conference, both in May 2011. Further and final data analysis was led by Veronika Northrup and Karol Katz from the Yale Center for Analytical Sciences though I had an active role in the preparation of the data and did directly undertake several of the statistical analyses.

- **Identifying and assisting future collaborations**

I acted to identify and assist future collaborations between KBTH (University of Ghana Medical School) and Yale School of Medicine, particularly in the study of virological failure and resistance patterns. I assisted with the storage of extra study samples to be used in the future to study the development of HIV resistance patterns in this longitudinal cohort at KBTH. Additional samples were collected for all participants identified as treatment failures and additional samples were stored. During my work

period in Accra, several samples were processed and evaluated for resistance, and a collaboration was established for future study with continued support from the Doris Duke Clinical Research Fellowship program.

- **Day-to-day team leadership and study implementation**

While in Ghana, I was in charge of the day-to-day implementation of the study protocol and the management and protection of all study data. These responsibilities included cooperation with all study personnel, coordination of team meetings and procurement of laboratory supplies.

4. RESULTS

A. Study Participants

There were 96 clinic patients initially recruited for enrollment into this study, as shown in Figure 4. Nine patients were subsequently excluded from enrollment and inclusion in the study. One child was identified several weeks after initial recruitment to be starting a second-line ART regimen and was therefore excluded. Sadly, this patient died several months after starting the second-line regimen, likely due to an abdominal lymphoma. One child upon further laboratory investigation was found to be HIV-negative and thereafter excluded from the study. Seven patients were recruited in the first year of the study before ART initiation. Study personnel continued following these patients to monitor the timing of ART initiation. However, by the end of data collection for this project, the seven children were still not yet on ART and were thereby excluded.

Eighty-seven participants were enrolled into the study and at the time data collection was closed for the purposes of this study, 63 participants had a complete data set that included baseline laboratory values and follow-up laboratory values at least 24 weeks after ART initiation. At least 24 weeks of follow-up data was needed in order to evaluate the effectiveness of ART based on WHO criteria. These 63 participants were included in the full data analysis and therefore are the participants described in Table 1. Twenty-four of the enrolled participants were excluded from the data analysis due to missing data. Data was missing for these participants due to several challenges. Some

initial blood samples were not collected in the designated window surrounding ART initiation, which was +/- three months before or after ART initiation. Additionally, some blood samples were affected by technical difficulties in specimen processing. A small number of samples were misplaced in the processing and mistakes were not discovered until after the baseline window had past. Other participants had not been enrolled for at least 24 weeks and therefore follow-up sample collection was not yet possible. For these enrolled participants who were awaiting collection of follow-up data, samples will be collected at the appropriate time for further research purposes related to a broader research context.

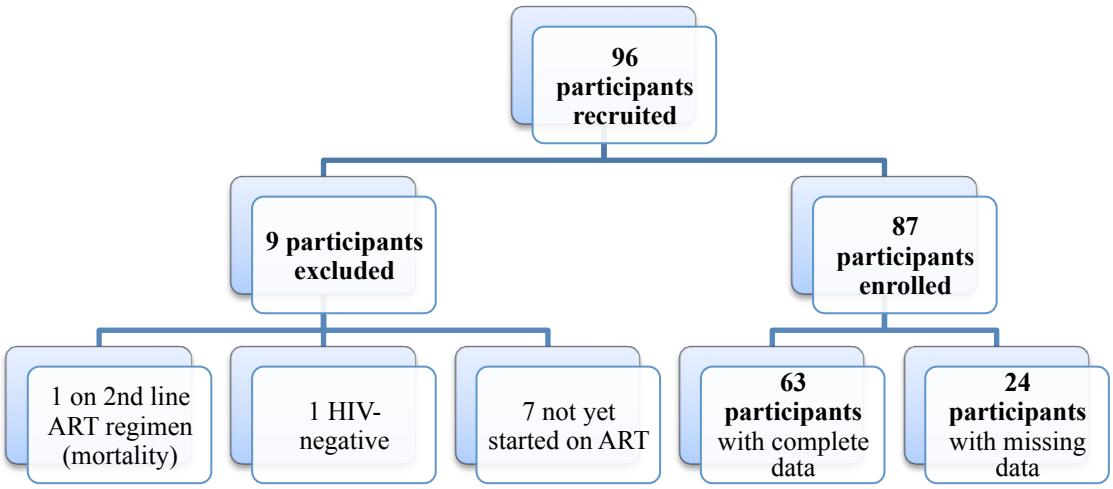

Figure 4. Enrollment flow chart

Table 1 illustrates the demographic characteristics of the study population (n = 63). There were slightly more male participants than female (57.1% versus 42.9%), and the mean age of all participants at ART initiation was 6.4 years (SD = 3.1). The youngest participant included in this data analysis was 1.2 years at the time of ART initiation and

the oldest was 12.8 years. The mean number of days between HIV diagnosis and ART initiation was 294 days (SD = 494), representing a period of approximately 10 months from the time that a child was found to be HIV-positive and when he/she started ART.

At the time of the initial interview, the participants or guardians were asked to report who the primary caregiver for the child was. For forty participants (63.5%), the primary caregiver was a parent, either the child's mother or father. However, for 36.5% of the participants the primary caregiver was someone other than a parent. The "other" caregivers included grandmother, aunt, uncle, an older sibling or a friend of a parent.

The various modes, and suspected modes, of HIV transmission are displayed in the table. A mode of "maternal" transmission was determined when the mother of the participant was known to be HIV-positive. In our study, this was the majority of the cases, with 69.8% of all participants having an HIV-positive mother. The mode of transmission was deemed "suspected maternal" when the mother's HIV status was unknown due to lack of testing or the mother was deceased. In these cases, due to the published research on the overwhelming majority of new pediatric HIV cases being caused by maternal transmission, it was decided to deem these cases "suspected maternal". The mode of transmission was deemed "unknown" in cases in which the mother's status was known to be HIV-negative. There were four such cases (7.9%) in our study population.

Participants and guardians were asked about PMTCT services received by mother and child during and after delivery. Only one participant (1.6%) had documentation showing receipt of PMTCT services for mother and child during and after delivery. The majority of participants (88.9%) reported no PMTCT services received by mother or

child and 9.5% of the participants did not know whether PMTCT services were received. Most of the cases in which PMTCT services were unknown were in cases of deceased mothers where a guardian who did not know the full details of the child's birth history was caring for the child.

A significant proportion of the study participants was orphaned, presumably due in large part to HIV/AIDS. 42.8% of participants had lost one or both of their parents at the time of enrollment. As expected, the majority of participants had at least one parent with known HIV infection (74.6%). The rest of the participants did not know the status of either one of their parents. While the rates amongst participants' parents were expectedly high, the prevalence of HIV among participants' siblings was low. Only four participants (6.3%) had siblings with known HIV infection. More than one-quarter (27.0%) knew that all siblings were HIV-negative and 27.0% had no siblings.

The low levels of disclosure reflect both the young average age of the study participants and the social stigma still surrounding the diagnosis of HIV. Partial disclosure was defined as the child knowing that he/she was sick and receiving treatment, but did not know the illness was HIV. Complete disclosure was defined as knowledge of illness and HIV. Few participants in our study even knew they were sick. 90.5% had no knowledge of their illness or HIV.

The predominant ART regimen consisted of two nucleoside reverse transcriptase inhibitors (NRTI) and one non-nucleoside reverse transcriptase inhibitor (NNRTI). The most popular regimen used (79.4%) was lamivudine (an NRTI, abbreviated 3TC), zidovudine (an NRTI, abbreviated AZT) and efavirenz (an NNRTI, abbreviated EFV). In 17.4% of cases, nevirapine (an NNRTI, abbreviated NVP) was substituted for EFV. One

patient was on a regimen of 3TC, AZT and abacavir (an NRTI, abbreviated ABC) while the remaining patient was taking 3TC, EFV and ABC. The use of ABC was mainly due to interactions with anti-TB medications.

The WHO utilizes a system to stage the severity of HIV disease based on clinical findings and evidence of various opportunistic infections. Stage I is deemed asymptomatic disease, stage II is mild disease, stage III is advanced and stage IV is severe. ART initiation is recommended at stages III and IV regardless of laboratory results. Therefore it was not unexpected that the large majority of our participants (79.5%) were in stage III or IV at the time of ART initiation.

One concomitant infection for which we screened through the interview and chart review was TB. At the time of ART initiation, 39.7% were previously or currently infected with TB. Throughout the follow-up period, we monitored and documented any new diagnoses of TB and used this variable in the data analysis to evaluate any effect that this common concomitant infection may have on the effectiveness of ART in our study population.

Height and weight were recorded at every clinic visit. Z scores were generated and demonstrate the number of standard deviations away from the mean for age-adjusted worldwide population. A significant proportion of our participants were below two standard deviations from the mean in height (38.0%) and weight (47.3%). Body mass index (BMI) was calculated using the height and weight of participants. The mean BMI for all participants was 14.8.

Table 1. Baseline Demographic Characteristics (n = 63)	
Characteristic	**Participants (%)**
Gender	
Female	27 (42.9)
Male	36 (57.1)
Age at ART initiation	
0 – 2 years	6 (9.5)
2 – 5 years	18 (28.6)
≥ 5 years	39 (61.9)
Primary caregiver	
Parent (mother / father)	40 (63.5)
Other (grandparent, other relative)	23 (36.5)
Mode of HIV transmission (n = 62)	
Maternal	44 (69.8)
Suspected maternal	14 (22.2)
Unknown	4 (7.9)
PMTCT treatment received	
Yes	1 (1.6)
No	56 (88.9)
Unknown	6 (9.5)
Parental living status	
Known both alive	36 (57.1)

Known one alive	21 (33.3)
Both died or unknown	6 (9.5)
Parental HIV status	
Both parents with HIV	16 (25.4)
One parent known with HIV	31 (51.7)
Both parents unknown	16 (25.4)
Siblings with known HIV	
Yes	4 (6.3)
No	17 (27.0)
Unknown	25 (39.7)
No siblings	17 (27.0)
Disclosure to patient (n = 60)	
None	57 (90.5)
Partial	2 (3.2)
Complete	1 (1.6)
ART regimen	
Lamivudine, zidovudine, efavirenz	50 (79.4)
Lamivudine, zidovudine, nevirapine	11 (17.4)
Other	2 (3.2)
WHO stage at ARV initiation (n = 59)	
I	3 (5.1)
II	15 (25.4)
III	24 (40.7)

IV	17 (38.8)
Previous TB infection	
Yes	25 (39.7)
No	38 (60.3)
Height *z* score (n = 41)	
-2 or below	10 (38.0)
$-2 > z < 2$	30 (60.0)
2 or above	1 (2.0)
Weight *z* score (n = 55)	
-2 or below	26 (47.3)
$-2 > z < 2$	29 (52.7)
2 or above	0 (0.0)
BMI *z* score (n = 50)	
-2 or below	8 (16.0)
$-2 > z < 2$	42 (84.0)
2 or above	0 (0.0)

In Table 2, the average baseline laboratory characteristics are reported for all participants at baseline. As expected, the biomarkers for CD4+ T-lymphocytes were low (average absolute CD4 count of 474, CD4 cell percentage of 13.0). These biomarkers are currently used by the clinic physicians at KBTH for the timing of ART initiation based on WHO criteria. The HIV RNA viral load at baseline was found to be over 250,000 copies/ml, indicating robust active infection with high levels of viral replication. On

average, the study participants were anemic, having a mean hemoglobin count of 8.9 g/dl, which meets criteria for anemia in any age category.

Table 2. Baseline Laboratory Characteristics (n = 63)

Characteristic	Mean (S.D.)
Absolute CD4 cell count (cells/µl)	474 (434)
CD4 cell percentage	13.0 (8.7)
Viral load (copies/ml)	251323 (770457)
Hemoglobin (g/dl)	8.9 (2.5)
WBC[a] (10^3/µl)	7.3 (4.0)
TLC[b] (10^3/µl)	3.8 (3.0)

[a] WBC = White Blood Cell count; [b] TLC = Total Lymphocyte Count

Further details on the baseline laboratory characteristics are displayed in Tables 3 through 5. Table 3 shows the differences in absolute CD4 cell count based on gender, age, WHO stage and TB diagnosis at or before ART initiation. There was a significant and expected difference in absolute CD4 count with age ($p<0.05$). As stated in the introduction, there is a documented physiological decline in the absolute number of CD4+ T-lymphocytes as the immune system matures and this number stabilizes after the age of 5 or 6 years. Therefore, we expected to see a significantly lower absolute CD4 count in the age group 5 years and older. There was also a significant and expected correlation between a lower absolute CD4 cell count and more advanced WHO stage at ART initiation ($p<0.05$). This correlation was expected because a lower absolute CD4 cell count indicates greater immune suppression, making the individual more susceptible

to opportunistic infections. The WHO stage is a measure of clinical severity based on clinical findings and opportunistic infections, with more advanced stages associated with more opportunistic infections. Therefore, it would be expected that individuals with greater immune suppression due to HIV disease, based on absolute CD4 cell counts, would have greater disease severity, based on more advanced WHO staging, at the time of ART initiation. There were no significant differences in absolute CD4 cell count based on gender or based on past or present TB infection.

Table 3. Baseline Laboratory Characteristics – Absolute CD4 cell count (n = 63)

Characteristic	**Absolute CD4+ T-lymphocyte count** (cells/µl)			
	n	Mean	SD	p value
Gender				
Female	26	434.5	365.5	0.270
Male	37	555.4	461.0	
Age at ART initiation				
0 – 2 years	7	712.4	431.2	0.013
2 – 5 years	18	689.1	539.3	
≥ 5 years	38	380.4	314.3	
WHO stage at ART initiation (n = 58)				
I	4	877.3	629.7	0.040
II	13	498.5	244.5	

III	23	525.2	409.3	
IV	18	320.1	281.1	
Previous TB infection (n = 62)				
Yes	24	585.0	501.7	0.293
No	38	467.9	367.2	

The differences in CD4 cell percentage are shown in Table 4. There were no significant statistical differences in mean CD4 cell percentage based on gender, age, WHO stage at baseline, or past or present TB diagnosis. However, there was an observed difference based on gender with females having a lower CD4 cell percentage at baseline, but this difference did not reach statistical significance (p=0.057).

Table 4. Baseline Laboratory Characteristics –

CD4 cell percentage (n = 63)

Characteristic	**CD4+ T-lymphocyte cell percentage**			
	n	Mean	SD	p value
Gender				
Female	26	11.6	8.1	0.057
Male	37	15.8	9.0	
Age at ART initiation				
0 – 2 years	7	14.9	7.6	0.461
2 – 5 years	18	15.5	9.1	
≥ 5 years	38	13.2	9.0	

WHO stage at ART initiation (n = 58)				
I	4	21.9	5.7	0.161
II	13	15.5	6.9	
III	23	13.6	9.1	
IV	18	11.6	9.0	
Previous TB infection (n = 62)				
Yes	24	13.4	8.7	0.563
No	38	14.8	8.9	

The differences in HIV RNA viral load (copies/ml) are displayed in Table 5. The data shows that there was a significantly higher viral load in females at the time of ART initiation compared in males ($p<0.05$), which perhaps correlated with the observed trend towards a lower CD4 cell percentage at baseline for females. There was also a significantly higher viral load found in patients younger than 2 years compared to the older groups ($p<0.05$). No significant differences were found in HIV viral load at baseline based on either disease severity measured by WHO staging or past or present TB infection.

Table 5. Baseline Laboratory Characteristics – HIV viral load (n = 51)				
Characteristic	**HIV viral load** (copies/ml)			
	n	Mean	SD	p value
Gender				
Female	22	926414	2182836	0.037
Male	29	58397	136822	
Age at ART initiation				
0 – 2 years	4	2360665	4719557	0.021
2 – 5 years	13	157925	210430	
≥ 5 years	34	311146	892797	
WHO stage at ART initiation				
I	3	113386	196253	0.446
II	12	62805	141282	
III	22	343407	821244	
IV	14	958991	2624576	
Previous TB infection				
Yes	20	344480	891319	0.736
No	31	489840	1776312	

B. Effectiveness of first-line ART regimens

The effectiveness of the first-line ART regimens was evaluated using the WHO criteria for treatment failure, immunological failure based on both absolute CD4 cell count or CD4 cell percentage, and virological failure based on viral load. As previously explained, a proportion of our enrolled participants were missing data. Of the 87 enrolled participants, 24 participants were missing CD4 data either at baseline or follow-up data. The flow chart indicating our data collection is shown in Figure 5, based on the collection of CD4 data from enrolled participants. This flow chart also indicates the number of participants meeting the criteria for immunological failure. The participants who did not meet these criteria were deemed to be receiving effective treatment. In our study, there were three participants (4.8%) who met the criteria for immunological failure after at least 24 weeks of ART, and therefore for 95.2% of our study population the first-line ART was effective for at least the first 24 weeks.

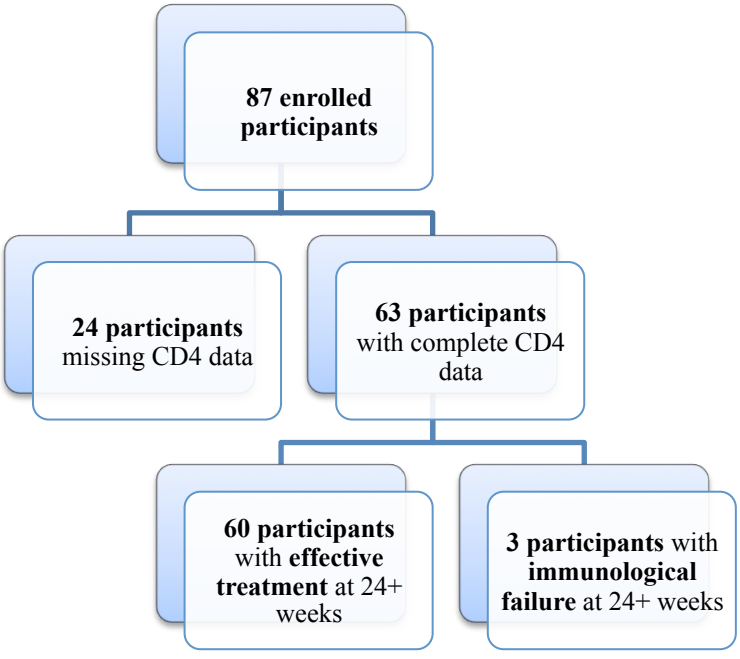

Figure 5. CD4 data collection flow chart with **immunological failures**

As shown in Figure 6, there were 53 participants with complete data to evaluate treatment effectiveness based on viral load data. There was an increased number of participants with missing data as compared to CD4 data due to technical difficulties with the viral load equipment leading to several samples being collected and stored, but not analyzed. These samples will continue to be stored and may be analyzed at a later date for further research studies based on the protocol. Based on the WHO definition of virological failure, there were nine participants (17.0%) who were determined to be virological failures.

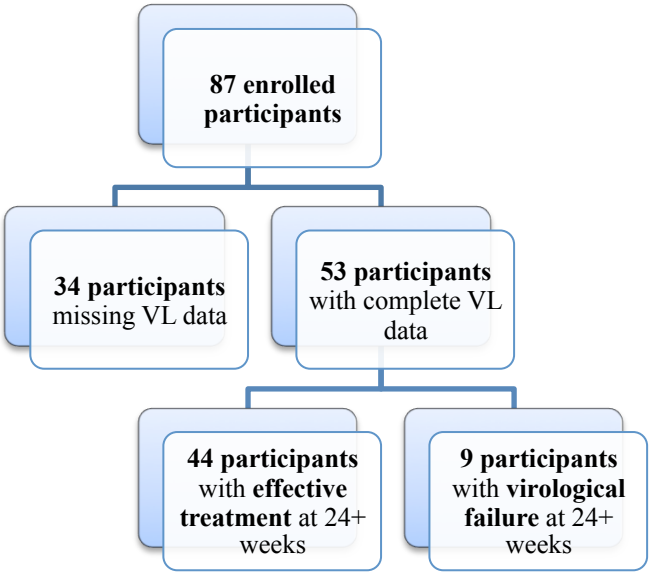

Figure 6. Viral load (VL) data collection flow chart – **Virological failures**

As explained in the Methods section, all participants with a viral load greater than 5,000 copies/ml after at least 24 weeks on ART would be seen in clinic for clinical review, assessment of compliance and counseling regarding medication adherence. At the subsequent visit, repeat blood samples were collected and laboratory testing was repeated. The purpose of this review and repeat laboratory testing was to gather further information to indicate whether HIV resistance or non-compliance was the cause for

reduced viral suppression. There were twelve participants (22.6%) who had viral load measurements greater than 5,000 copies/ml after at least 24 weeks of ART. For seven of these participants, the high viral load measurement was the most recent measurement and therefore they were deemed virological failures for purposes of this data analysis. Five of the twelve participants had subsequent laboratory testing. Three of the five participants (60%) had repeat viral load measurements greater than 5,000 copies/ml. The other two participants (40%) had subsequent viral load measurements that fell to undetectable levels following the adherence counseling and monitoring.

The virological response to the first-line ART was dramatic and is depicted in the graphs of Figures 7 and 8. In Fig. 7, the short-term response to ART based on viral load is depicted in a spaghetti plot using a log-scale. Each line represents an individual participant and data points up to 30 weeks following ART initiation. Overall, the viral loads for our study participants decreased steeply over the short-term period following ART initiation. The individuals for whom the viral load increased over this period were identified as virological failures and can be seen in Figure 7. In Fig. 8, the longitudinal response of viral load over the entire study period (up to 24 months) is depicted as mean viral loads for all individuals with data points. The mean viral load response dropped exponentially between ART initiation and the second clinic visit at approximately 6 months follow-up, demonstrating the immediate response of participants to first-line ART regimens. The mean viral load appeared to stabilize after approximately the 6-months follow-up visit and remain low.

Additionally, the proportion of participants with undetectable viral load reflected a strong response. At the second clinic visit, at approximately 6 months, 50% (n=22) of

participants had an undetectable viral load, defined as less than 400 copies/ml. This proportion grew significantly at the third clinic visit, at approximately 12 months following ART initiation, to 76.7% of participants (n=23) and appeared to stabilize at the 18-month (68.4%, n=13) and 24-month (71.4%, n=5) follow-up visits.

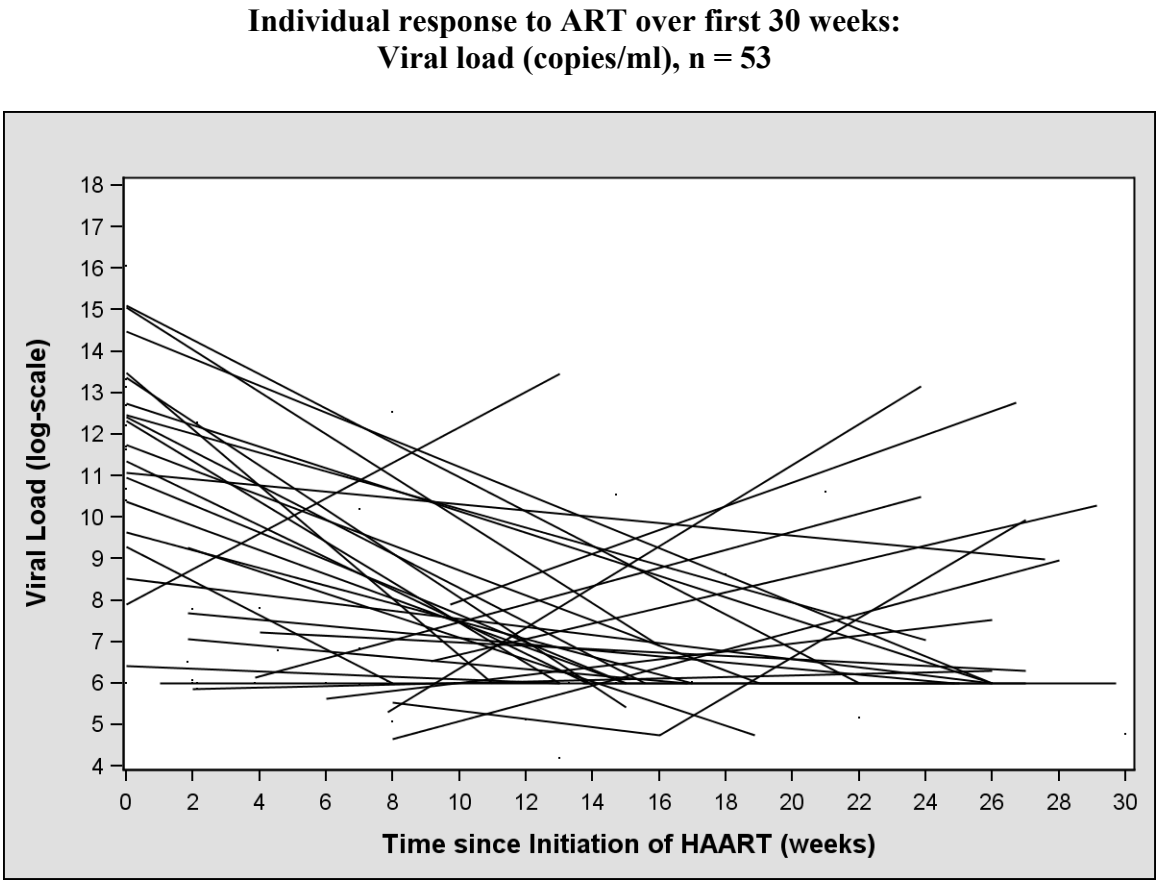

Figure 7. Short-term response to ART based on viral load data (copies/ml) from baseline and up to 30 weeks following ART initiation. Each line represents an individual participant.

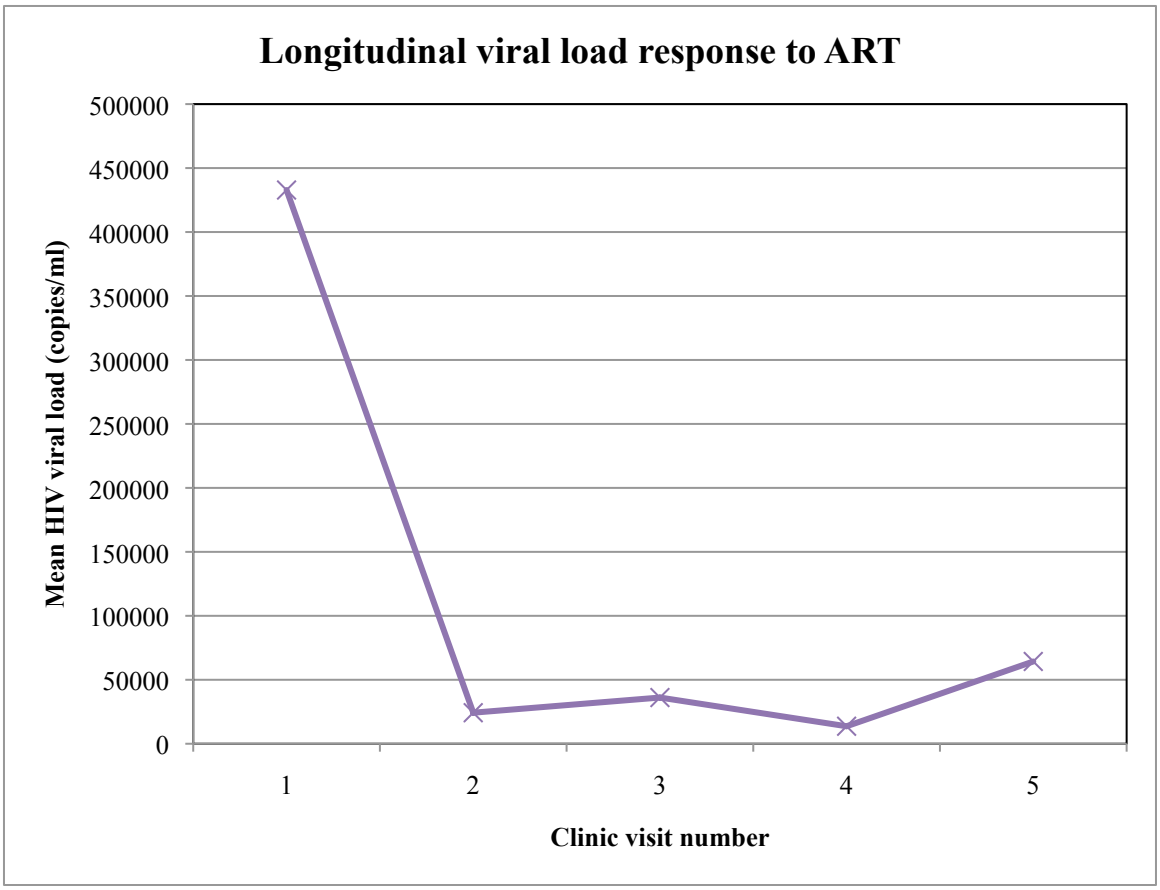

Figure 8. Trends in the HIV viral load for study participants over the first 5 visits. The mean HIV viral load (copies/ml) is plotted for each clinic visit. The table below contains the number of participants included in the analysis at each clinic visit.

Visit	1 (0 months)	2 (6 months)	3 (12 months)	4 (18 months)	5 (24 months)
N	51	44	30	19	7

The difference in immunological (n = 3) versus virological failures (n = 9) was expected. Based on previous research, as effectiveness of ART wanes, a rise in the viral load is observed first, followed, sometimes months later, by a decline in the absolute CD4 cell count and CD4 cell percentage. Analyses were conducted to evaluate the agreement between immunological failure and virological failure determination. Of the three immunological failures, two were also determined to be virological failures while one

participant was found to be an immunological failure but not a virological failure. Conversely, of the nine virological failures, two participants were also immunological failures and seven participants were not. These data are reported in Table 6 below. Table 6 shows the agreement between the determinations of failures based on immunological and virological failure. For this analysis, only participants that had both complete CD4 data and complete viral load data were included. The kappa statistic was calculated to be 0.27 (95% CI: -0.07, 0.61).

Table 6. Agreement between failure criteria (n = 53)

	Immunological failure		
Virological failure	Failure	No failure	**Total**
Failure	2	7	**9**
No failure	1	43	**44**
Total	**3**	**50**	**53**

Kappa = 0.27 (95% CI: -0.07, 0.61)

The effectiveness of the first-line ART regimens was also demonstrated by the mortality and toxicity data. In our 63 participants with complete CD4 data, there were no mortalities (0%) in 24 or more weeks of follow-up. Three participants switched ARV regimen (4.7%). Two participants switched due to toxicity, both secondary to nevirapine (generalized rash and unspecified toxicity). One participant switched regimens due to interaction with anti-TB medications.

Predictor variables for immunological, virological and overall treatment failure were assessed. Associations between baseline laboratory and demographic

characteristics with immunological failure are demonstrated in Tables 7 and 8. One predictor variable identified for immunological failure was baseline absolute CD4 cell count. A lower absolute CD4 cell count was significantly associated with immunological failure ($p<0.05$). However, baseline CD4 cell percentage was not found to be associated with immunological failure ($p=0.242$). Routine biomarkers such as hemoglobin, lymphocyte count and white blood cell count did not predict immunological failure at baseline.

When stratified by three age groups and gender, there were no significant differences across the groups. All participants with immunological failure had advanced (stage III) or severe (stage IV) HIV disease at ART initiation, while 32.2% of participants with effective treatment started ART with asymptomatic (stage I) or mild (stage II) disease. These differences did not reach statistical significance ($p=0.130$). A notable difference was observed in the different ART regimens. All participants with immunological failure had an EFV-based regimen, which was the most popular regimen for all participants (79.4%). However, the difference did not reach statistical significance ($p = 0.66$).

Participants whose parents were both deceased were more likely to fail treatment immunologically than participants with at least one parent alive ($p<0.05$). Other variables such as caregiver and the HIV status of parents, did not predict the effectiveness of ART using CD4 criteria. Furthermore, past or present TB infection did not predict ART effectiveness.

Table 7. Effectiveness of first-line ART regimens and associations with baseline laboratory characteristics for IMMUNOLOGICAL FAILURES, n = 63			
Baseline Characteristic	**Failed Treatment (SD), n = 3**	**Effective Treatment (SD), n = 60**	**p value**
Mean age at ART initiation (years)	7.5 (3.4)	6.4 (3.2)	0.563
Time from HIV diagnosis to ART initiation (days)	45 (5.7)	261 (391)	0.222
Mean absolute CD4 cell count (cells/µl)	30.3 (9.9)	499.9 (435.6)	0.041
Mean CD4 cell percentage	7.0 (10.4)	13.3 (8.7)	0.242
Mean viral load (copies/ml)	8680 (12182)	267867 (794118)	0.761
Mean WBC (10^3/µl)	8.4 (1.5)	7.3 (4.1)	0.210
Mean Hgb (g/dl)	9.5 (0.1)	8.8 (2.6)	0.928
Mean TLC (10^3/µl)	3.7 (2.0)	3.8 (3.2)	0.937
Mean BMI (kg/m^2)	13.9 (2.0)	14.8 (1.7)	0.331

Table 8. Effectiveness of first-line ART regimens and associations with baseline characteristics for IMMUNOLOGICAL FAILURES, n = 63				
Baseline Characteristic	**Failed Treatment**	**Effective Treatment**	**Total population**	**p value**
	n (%)	**n (%)**	**n (%)**	
Age at ART initiation (years)				
0 – 2 years	0 (0)	6 (10)	6 (9.5)	
2 – 5 years	1 (33.33)	17 (28.33)	18 (28.6)	0.81
≥ 5 years	2 (66.67)	37 (61.67)	39 (61.9)	
Gender				
Female	1 (33.33)	26 (43.33)	27 (42.9)	1.00
Male	2 (66.67)	34 (56.67)	36 (57.1)	
WHO staging (n = 59)				
Stage I	0 (0)	3 (5.36)	3 (5.1)	0.13
Stage II	0 (0)	15 (26.79)	15 (25.4)	
Stage III	1 (33.33)	23 (41.07)	24 (40.7)	
Stage IV	2 (66.67)	15 (26.79)	17 (38.8)	
ART regimen				
3TC/AZT/EFV[a]	3 (100)	47 (78.33)	50 (79.4)	0.66
3TC/AZT/NVP[b]	0 (0)	11 (18.33)	11 (17.4)	
Other	0 (0)	2 (3.34)	2 (3.2)	
Caregiver				
Mother / Father	1 (33.33)	39 (65)	40 (63.5)	0.55

Other	2 (66.67)	21 (35)	23 (36.5)	
Parent status				
Both died / Unknown	2 (66.67)	4 (6.67)	6 (9.5)	0.03
Known one alive	0 (0)	21 (35.0)	21 (33.33)	
Known both alive	1 (33.33)	35 (58.33)	36 (57.1)	
Parent HIV status				
Both parents unknown	1 (33.33)	15 (25.0)	16 (25.4)	0.41
One parent with HIV	0 (0)	31 (51.67)	31 (51.7)	
Both parents with HIV	2 (66.67)	14 (23.33)	16 (25.4)	
Previous TB infection				
Yes	2 (66.67)	23 (38.33)	25 (39.7)	0.56
No	1 (33.33)	37 (61.67)	38 (60.3)	

[a] = lamivudine (3TC), zidovudine (AZT), efavirenz (EFV); [b] = lamivudine (3TC), zidovudine (AZT), nevirapine (NVP)

The analysis of predictor variables for virological failures, displayed in Tables 9 and 10, showed that the length of time between HIV diagnosis and ART initiation was significantly associated with treatment effectiveness. Participants with a shorter time period between diagnosis and treatment were more likely to fail treatment ($p<0.05$). However, baseline absolute CD4 cell count and CD4 cell percentage did not predict treatment effectiveness for those determined to be virological failures. As with immunological failures, none of the routine biomarkers (hemoglobin, WBC, TLC) showed any significant association with treatment effectiveness.

Different age groups or gender did not predict virological failure in this study population. There were virological failures found in all the various ART regimens,

which differs from the findings for immunological failures, which were found only for the 3TC/AZT/EFV regimen. The household demographics (parent living status, parent HIV status and primary caregiver) also did not predict virological failure in this population. Additionally, past or present TB infection was not associated with virological failure.

Table 9. Effectiveness of first-line ART regimens and associations with baseline laboratory characteristics for VIROLOGICAL FAILURES, n = 53

Baseline Characteristic	**Failed Treatment (SD), n = 9**	**Effective Treatment (SD), n = 44**	**p value**
Mean age at ART initiation (years)	7.1 (3.9)	6.3 (3.1)	0.452
Time from HIV diagnosis to ART initiation (days)	68.5 (81.0)	295.5 (430.8)	0.037
Mean absolute CD4 cell count (cells/µl)	344.9 (428.0)	508.6 (463.1)	0.309
Mean CD4 cell percentage	11.9 (9.2)	12.6 (8.9)	0.810
Mean viral load (copies/ml)	9261 (22277)	300976 (838818)	0.188
Mean WBC (10^3/µl)	9.5 (7.1)	7.0 (3.3)	0.739
Mean Hgb (g/dl)	9.5 (0.4)	9.0 (2.7)	0.965
Mean TLC (10^3/µl)	4.9 (6.1)	3.7 (2.1)	0.790
Mean BMI (kg/m^2)	14.5 (1.7)	14.8 (1.8)	0.676

Table 10. Effectiveness of first-line ART regimens and associations with baseline characteristics for VIROLOGICAL FAILURES, n = 53				
Baseline Characteristic	**Failed Treatment**	**Effective Treatment**	**Total population**	**p value**
	n (%)	**n (%)**	**n (%)**	
Age at ART initiation (years)				
0 – 2 years	2 (22.22)	3 (6.82)	5 (9.4)	
2 – 5 years	1 (11.11)	14 (31.82)	15 (28.3)	0.23
≥ 5 years	6 (66.67)	27 (61.36)	33 (66.7)	
Gender				
Female	2 (22.22)	19 (43.18)	21 (43.20)	0.29
Male	7 (77.78)	25 (56.82)	32 (56.80)	
WHO staging (n = 50)				
Stage I	0 (0)	3 (7.32)	3 (6.0)	0.39
Stage II	3 (33.33)	11 (26.83)	14 (28.0)	
Stage III	2 (22.22)	17 (41.46)	19 (38.0)	
Stage IV	4 (44.44)	10 (24.390	14 (28.0)	
ART regimen				
3TC/AZT/EFV[a]	6 (66.67)	36 (81.82)	42 (79.20)	0.38
3TC/AZT/NVP[b]	2 (22.22)	7 (15.89)	9 (16.90)	
Other	1 (11.11)	1 (2.27)	2 (3.80)	
Caregiver				

Mother / Father	5 (55.56)	29 (65.91)	34 (64.2)	0.7
Other	4 (44.44)	15 (34.09)	19 (35.9)	
Parent status				
Both died / Unknown	3 (33.33)	3 (6.82)	6 (11.3)	0.12
Known one alive	2 (22.22)	16 (36.36)	18 (36.4)	
Known both alive	4 (44.44)	25 (56.82)	29 (54.7)	
Parent HIV status				
Both parents unknown	1 (11.11)	11 (25.0)	12 (22.6)	0.24
One parent with HIV	4 (44.44)	21 (47.73)	25 (47.2)	
Both parents with HIV	4 (44.44)	12 (27.27)	16 (30.2)	
Previous TB infection				
Yes	2 (22.22)	17 (38.64)	19 (35.8)	0.46
No	7 (77.78)	27 (61.36)	34 (64.2)	

[a] = lamivudine (3TC), zidovudine (AZT), efavirenz (EFV); [b] = lamivudine (3TC), zidovudine (AZT), nevirapine (NVP)

Predictor variables were analyzed for all participants determined to be failures based on either immunological or virological criteria (all failures) and results are depicted in Tables 11 and 12. There was one additional participant included in these analyses (n = 64) because that participant had incomplete CD4 data but complete viral load data. For all participants that failed treatment, a shorter interval between HIV diagnosis and ART initiation was found to be significantly associated with treatment failure (failure = 294.1 days, SD=493.6; effective = 332.9 days, SD=523.7, p = 0.014). Additionally, having both parents with HIV was found to be significantly associated with higher rates of treatment failure compared to participants with one parent with HIV or both parents with

unknown HIV status (p = 0.05 across groups). All other baseline characteristics, including all laboratory-based details, age, gender, WHO staging and previous TB infection, were not significantly associated with treatment failure. Different ART regimens were not significantly associated with overall treatment failures.

Table 11. Effectiveness of first-line ART regimens and associations with baseline laboratory characteristics for ALL FAILURES, n = 64

Baseline Characteristic	**Failed Treatment (SD), n = 10**	**Effective Treatment (SD), n = 54**	**p value**
Mean age at ART initiation (years)	6.4 (3.1)	6.4 (3.1)	0.69
Time from HIV diagnosis to ART initiation (days)	294.1 (493.6)	332.9 (523.7)	0.014
Mean absolute CD4 cell count (cells/µl)	473.7 (434.1)	504.1 (434.4)	0.13
Mean CD4 cell percentage	13.0 (8.7)	13.4 (8.6)	0.31
Mean viral load (copies/ml)	251323 (770457)	308299 (848813)	0.31
Mean WBC (10^3/µl)	7.3 (4.0)	6.8 (3.1)	0.29
Mean Hgb (g/dl)	8.9 (2.5)	8.8 (2.8)	0.85
Mean TLC (10^3/µl)	3.8 (3.0)	3.5 (2.0)	0.72
Mean BMI (kg/m^2)	14.8 (1.7)	14.8 (1.8)	0.66

Table 12. Effectiveness of first-line ART regimens and associations with baseline characteristics for ALL FAILURES, n = 64				
Baseline Characteristic	**Failed Treatment**	**Effective Treatment**	**Total population**	**p value**
	n (%)	**n (%)**	**n (%)**	
Age at ART initiation (years)				
0 – 2 years	2 (20.0)	4 (7.41)	6 (9.4)	
2 – 5 years	2 (20.0)	16 (29.63)	18 (28.1)	0.73
≥ 5 years	6 (60.0)	34 (62.96)	40 (62.5)	
Gender				
Female	2 (20.0)	25 (46.3)	27 (42.2)	0.17
Male	8 (80.0)	29 (53.7)	37 (57.8)	
WHO staging (n = 60)				
Stage I	0 (0)	3 (5.66)	3 (4.8)	0.69
Stage II	3 (30.0)	12 (22.64)	15 (23.8)	
Stage III	3 (30.0)	21 (39.62)	24 (38.1)	
Stage IV	4 (40.0)	13 (24.53)	17 (27.0)	
ART regimen				
3TC/AZT/EFV[a]	7 (70.0)	44 (81.48)	51 (79.69)	0.37
3TC/AZT/NVP[b]	2 (20.0)	9 (16.67)	11 (17.19)	
Other	1 (10.0)	1 (1.85)	2 (3.13)	
Caregiver				

Mother / Father	6 (60.0)	34 (63.0)	40 (62.5)	1.00
Other	4 (40.0)	20 (37.0)	24 (37.5)	
Parent status				
Both died / Unknown	3 (30.0)	4 (7.41)	7 (10.9)	0.20
Known one alive	2 (20.0)	19 (35.19)	21 (32.8)	
Known both alive	5 (50.0)	31 (57.41)	36 (56.3)	
Parent HIV status				
Both parents unknown	1 (10.0)	16 (29.63)	17 (26.6)	0.05
One parent with HIV	4 (40.0)	27 (50.0)	31 (48.4)	
Both parents with HIV	5 (50.0)	11 (20.37)	16 (25.0)	
Previous TB infection				
Yes	3 (30.0)	22 (40.74)	25 (39.1)	0.73
No	7 (70.0)	32 (59.26)	39 (60.9)	

[a] = lamivudine (3TC), zidovudine (AZT), efavirenz (EFV); [b] = lamivudine (3TC), zidovudine (AZT), nevirapine (NVP)

C. Use of absolute CD4 cell count

Analyses were conducted to evaluate both the short-term and long-term response to ART initiation based on absolute CD4 cell count and CD4 cell percentage. To analyze the short-term response to ART, spaghetti plots were constructed and are depicted below in Figures 9 and 10. In both figures, each line represents an individual participant. The data points included in these plots were baseline measurements and data points up to 30 weeks following ART initiation. The overall trajectories for absolute CD4 cell count

(Fig. 9) and for CD4 cell percentage (Fig. 10) in the short-term period appeared to be similar in slope and trend. The graphs illustrate a strong immunological response to first-line ART regimens. Individual declines in absolute CD4 count and CD4 cell percentage were observed and represent the immunological failures.

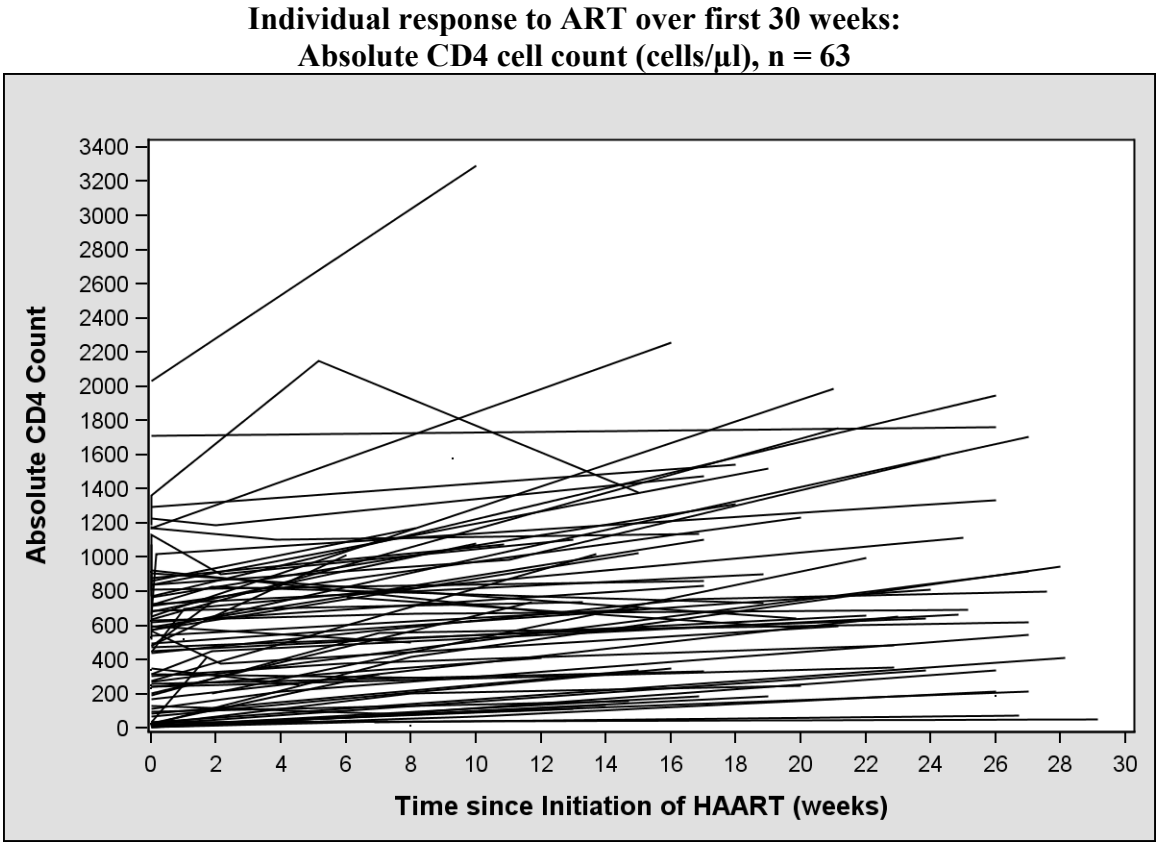

Figure 9. Short-term response to ART based on absolute CD4 cell count. Each line represents an individual participant.

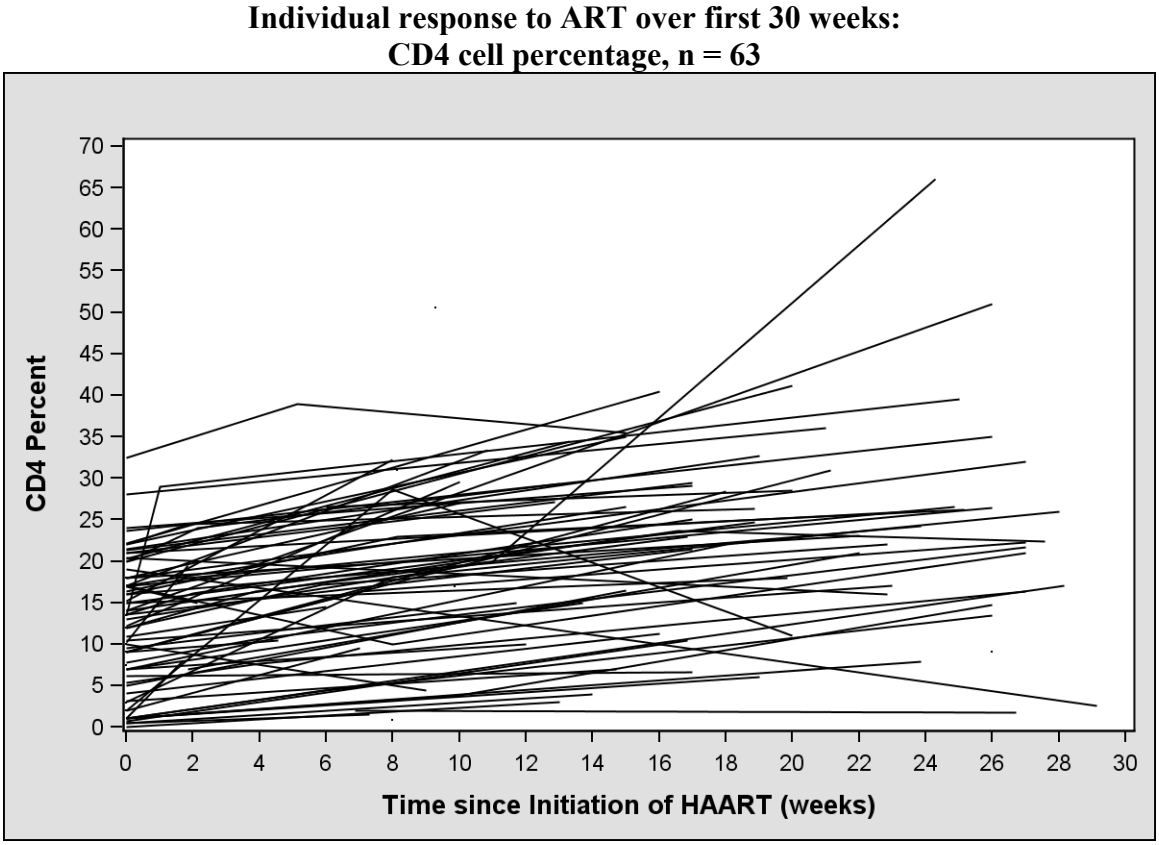

Figure 10. Short-term response to ART based on CD4 cell percentage. Each line represents an individual participant.

The longitudinal trends and trajectories of both absolute CD4 cell count and CD4 cell percentage were calculated, depicted in Table 13 and in graph form in Figures 11, 12 and 13. These trends were produced and analyzed based on mean absolute CD4 cell count and CD4 cell percentage at 6-month intervals following ART initiation. In our study, we followed participants as long as 24 months after ART initiation. However, the number of participants included in this trend analysis fell significantly after 12 months of follow-up.

The trends and trajectories of absolute CD4 cell count and CD4 cell percentage were observed to be descriptively similar. Both biomarkers increased 70-80% at six

months, doubled by 12 to 18 months after ART initiation and subsequently rose at a slower rate. Both absolute CD4 cell count and CD4 cell percentage appeared to stabilize similarly and plateau around the 18-month visit.

Table 13. Longitudinal trends in CD4 biomarkers on ART

Clinic visit number	**1 (0 mos)**	**2 (6 mos)**	**3 (12 mos)**	**4 (18 mos)**	**5 (24 mos)**
Mean absolute CD4 cell count, cells/µl [SD]	505.5 [425.3]	865.4 [525.5]	901.1 [558.5]	1069.3 [747.2]	1041.4 [269.3]
Mean CD4 cell percentage [SD]	14.1 [8.8]	25.4 [12.4]	26.0 [7.8]	27.7 [11.0]	29.1 [7.35]

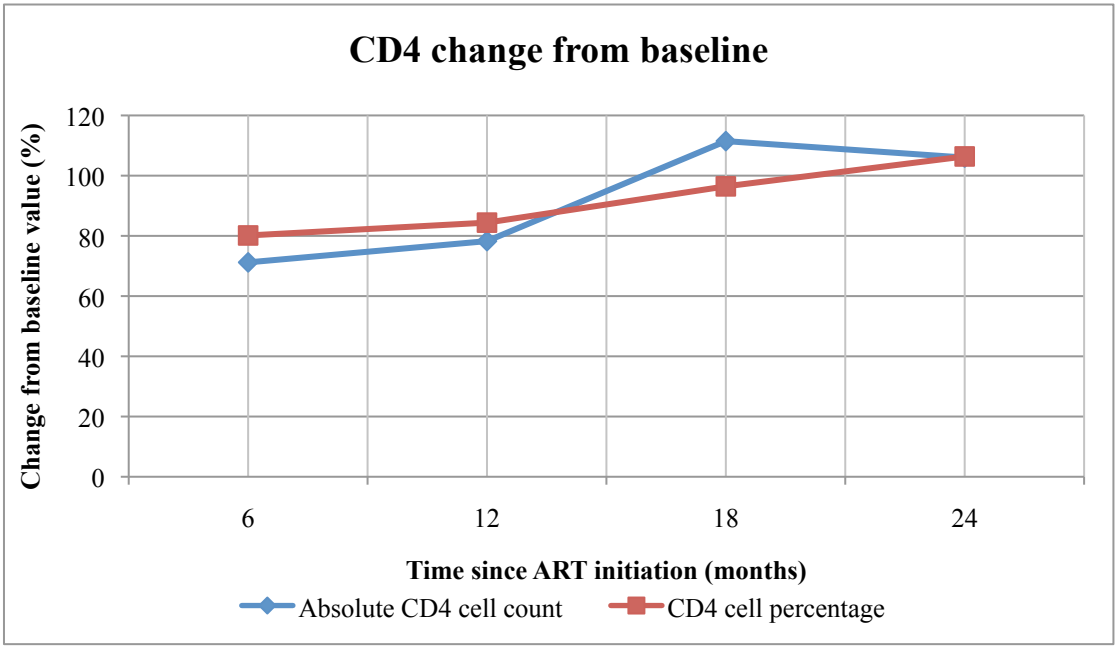

Figure 11. Trends in the absolute CD4 cell count and CD4 cell percentage as average percent changes from the participant's baseline.

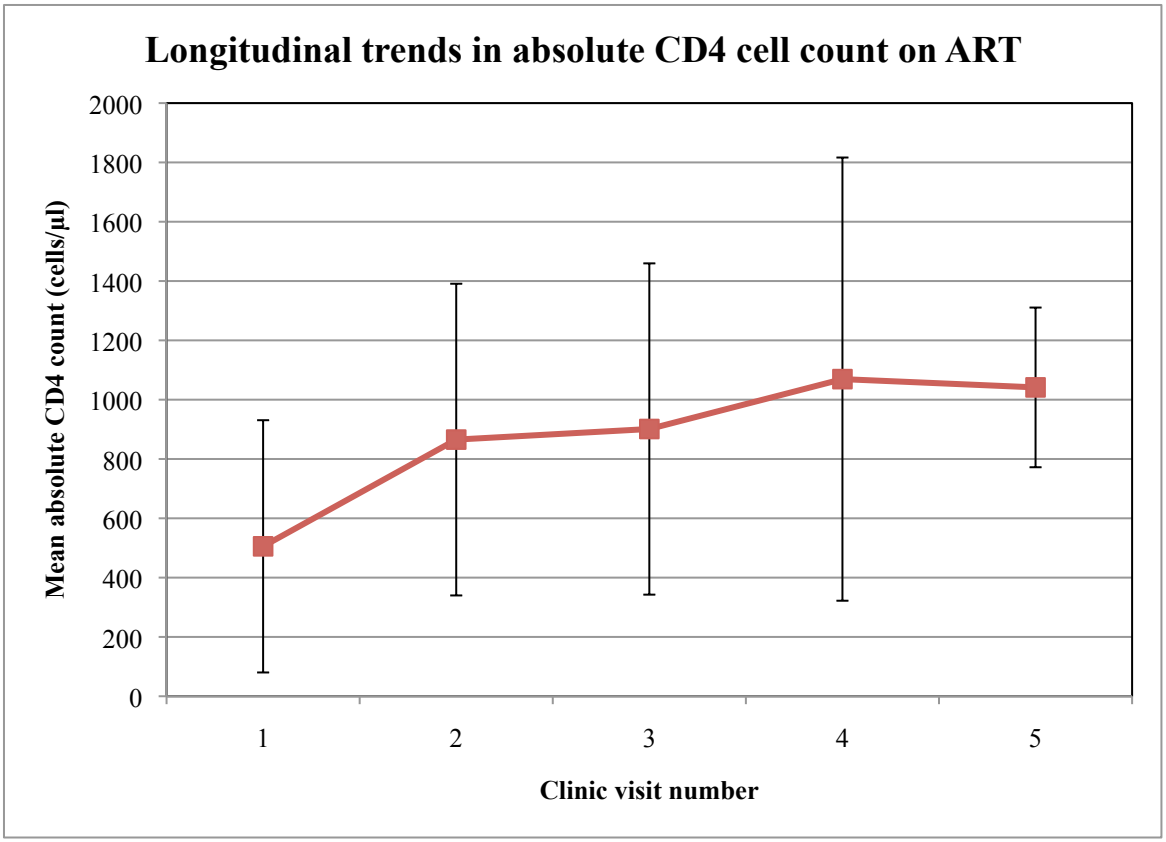

Figure 12. Trends in the absolute CD4 cell count for study participants over the first 5 visits. The mean absolute CD4 cell count (cells/µl) is plotted with standard deviation markers. The table below contains the number of participants included in the analysis at each clinic visit. Study visits and data collection took place every 4-6 months.

Visit	1 (0 months)	2 (6 months)	3 (12 months)	4 (18 months)	5 (24 months)
N	64	54	42	21	9

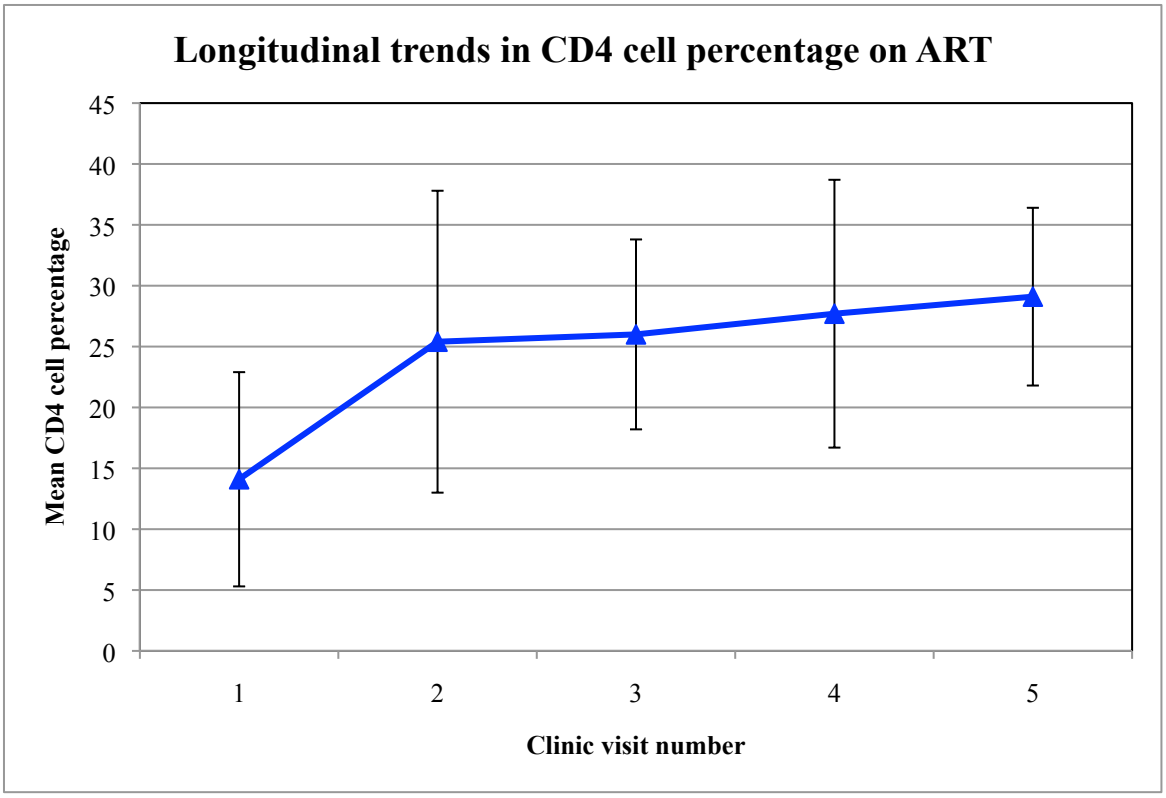

Figure 13. Trends in the CD4 cell percentage for study participants over the first 5 visits. The mean CD4 cell percentage is plotted with standard deviation markers. The table below contains the number of participants included in the analysis at each clinic visit. Study visits and data collection took place every 4-6 months.

Visit	1 (0 months)	2 (6 months)	3 (12 months)	4 (18 months)	5 (24 months)
N	64	53	42	21	9

5. DISCUSSION

A. Effectiveness of first-line ART regimens

The data from this study strongly demonstrate the effectiveness of the first-line regimen used at KBTH for HIV-infected children. For 83% of the study participants, the first-line ART regimen was effective. There were no deaths among the children with 24 weeks or more of follow-up. This level of mortality is comparable to previous studies. For example, in a large West Africa cohort, there was a 92% survival reported at 12 months[16]. In Cote d'Ivoire, there was a reported survival rate of 92% at 6 months following ART initiation[12]. Our study results are limited in number but the high survival rate strongly supports the effectiveness of the available first-line regimens.

Furthermore, the study data support the high tolerance of the first-line ART regimen in our population based on the low levels of toxicity, regimen switching and sustained viral load and CD4 response. There was a 4.7% rate of regimen switching and only two of the study participants switched because of drug toxicity (3.2%). These rates of regimen switching and toxicity are comparable to previous studies in the region and in other resource-limited settings globally. In Kenya, 10% of a pediatric cohort receiving first-line ART switched regimens due to drug toxicity[14] and a smaller percentage, 3.8%, was reported in a larger study conducted in multiple treatment centers in Africa and Asia[17]. The trajectories of viral load, absolute CD4 cell counts and CD4 cell percentages also support the durability of the first-line regimen in our population and follow-up

period. At 24 months of follow-up, 71% of our participants had complete viral suppression (<400 copies/ml), which was increased from the rate of 50% at six months. ART is life-long therapy and therefore our period of follow-up can only report preliminary evidence of durability, but the study findings are encouraging.

Both absolute CD4 cell counts and CD4 cell percentages showed a sustained response through 24 months of follow-up for all ages and genders. Additionally, the CD4+ T-lymphocyte cell response in our study population reproduced previously reported data from different participants within the same clinical setting. Renner et al. reported a doubling of absolute CD4 counts at six months for children at KBTH, a result that is similar to our data[22]. The replication of these previously published data validates the effectiveness of first-line regimens reported and described in this study.

The findings of this study supporting the effectiveness of first-line ART regimens in Accra, Ghana have important public health implications. These data show that the currently available regimens, most popularly a regimen of 3TC, AZT and EFV, are effective in a non-type B HIV genotype, and strongly support the scale-up and roll out of these current regimens to the broader pediatric HIV community in the region. Based on the low failure rates, it appears that circulating HIV strains and genotypes among HIV-infected children in Ghana are susceptible to current and available ART. Therefore, the increased production of low-cost formulations of the current regimen should be pursued. 3TC, AZT and EFV are all approved by the FDA, with some restrictions, for generic production.

The analysis of the first-line ART effectiveness also identified important predictors of treatment failure. The most consistent predictor of treatment failure was the

time interval between HIV diagnosis and ART initiation. The data show a significant association between shorter intervals from HIV diagnosis to ART initiation and overall treatment failure ($p<0.05$). The same association and direction were similarly reported for virological failure ($p<0.05$). For the immunological failure data, a shorter mean interval between HIV diagnosis and ART initiation was observed as well but did not reach statistical significance. The participants with immunological failure had a shorter period between diagnosis and treatment, 45 days on average (SD=5.7), compared to an average period of 261 days (SD=391) for participants with effective treatment ($p=0.222$). Increased failure rates for participants with shorter intervals between HIV diagnosis and ART initiation may be evidence that participants with acute, severe presentations to care may be more likely to fail treatment.

Age at ART initiation was not found to be significantly correlated with treatment effectiveness for the overall study population. However, the observed mean age was higher for individuals with immunological failure (failure = 7.5 years, SD=3.4; effective = 6.4 years, SD=3.2, $p=0.563$), as well as virological failure (failure = 7.1 years, SD=3.9; effective = 6.3 years, SD=3.1, $p=0.452$). As the patient population increases and the number of measurements increase, it will be worthwhile to watch to see if these associations become significant.

Both of these observed associations, interval between diagnosis and ART initiation and age at ART initiation, support the need for early HIV diagnosis and treatment. Similar to previous studies in the Africa context, the mean age at ART initiation was high (6.4 years [SD=3.1]), further supporting the need to improve methods of early diagnosis.

HIV screening for pregnant women is an issue beyond the scope of this study but given the high percentage of new pediatric HIV cases as a result of mother-to-child transmission, there may be a need for improved policies regarding HIV testing for pregnant women in Ghana. Currently, there is an opt-in policy in place by which all pregnant women are offered, by law, HIV testing and the mother decides whether she would like to be tested. An opt-out policy may be beneficial in order to identify a greater proportion of HIV-infected women and exposed infants, who regardless of transmission suffer increased mortality[58]. The low percentage of patients in this study who participated in PMTCT (1.6%) may indicate success of the PMTCT program for those enrolled. The success of prenatal screening and PMTCT in contrast to the high number of patients seen at the pediatric HIV clinic at KBTH and at older average ages suggests that ways to further improve early diagnosis should be considered and prioritized.

A lower baseline absolute CD4 cell count appears to be predictive of at least immunological failure ($p<0.05$) and a lower mean baseline absolute CD4 cell count was observed in participants with virological failures but did not reach statistical significance. When all treatment failures were analyzed together, this association was not found to be statistically significant. However, this association may indicate a difference in immune recovery and response for children starting ART at lower absolute CD4 counts. This result may indicate that participants in our population that had more severe immune suppression at the time of ART initiation are less likely to recover CD4 T-lymphocyte counts and improve on treatment.

One additional predictor variable that was reported to be significant for all treatment failures was parental HIV status. Participants whose parents were both known

to be HIV-positive had significantly higher rates of treatment failure (p=0.05). This association was not significant in the analyses for either immunological or virological failure. As many as 30% of participants that failed treatment had parents whose HIV status was unknown, which makes interpretation of this association difficult. However, the association with HIV-positive parents could indicate that HIV-positive parents may be struggling with their own health issues, hospitalizations, medication adherence, and may not be adequate supervisors of their child's health and adherence, which could contribute to treatment failure.

This study, through research funding and collaboration with the Virology Lab at KBTH, introduced the routine use of viral load testing to the Pediatric HIV/AIDS Care Program. The value of viral load testing was illustrated in the analysis of ART effectiveness. There were twelve participants found to have a viral load of greater than 5,000 copies/ml after at least 24 weeks of ART. Nine of the twelve participants (75%) were deemed virological failures based on subsequent viral load testing. Additionally, most (75%) of the increased viral load measurements were found at the 6-month follow-up visit. This observation reflected the potential value of viral load testing for identification of early treatment failures and resistance after six months of ART but decreased value for routine testing beyond six months.

The repeated use of viral load testing for participants with elevated viral loads highlighted the value of viral load testing for compliance. Reduced adherence is probably an underlying cause of failure in patients not initially infected with resistant virus. In our study, 40% of participants retested after adherence counseling and increased compliance monitoring had undetectable viral loads, indicating a significant proportion of

participants with a history concerning for poor compliance. Conversely, the 60% of participants retested had sustained rises in viral load and raised concerns for viral resistance. These results show the value of viral load testing even in the absence of resistance testing, as practiced in this study. The use of viral load testing, where available, could have value for treatment effectiveness, identification of disease resistance and non-compliance in resource-limited settings.

The analysis of agreement between immunological failures and virological failures (kappa = 0.27) shows poor agreement. Based on this data, the classification of treatment failure using CD4 data does not agree well with the classification of treatment failure using viral load data. The most likely explanation of the poor agreement is a delay between viral load response and CD4 response as a patient fails treatment. As shown in previous studies, virological failure may precede immunological failure by several months and therefore this poor agreement may be expected[40]. Despite this expectation and potential explanation, the poor agreement may indicate the value of having both CD4 and viral load capabilities when monitoring ART in children. Pediatric HIV programs in resource-limited settings should strive to access facilities capable of measuring both CD4 and viral load data to optimize the ability to detect treatment failures. However, this study is not powered for this analysis and therefore any interpretation is limited.

B. Use of absolute CD4 cell count

Based on this study's preliminary and descriptive analysis of absolute CD4 cell count and CD4 cell percentage as monitoring tools for ART in HIV-infected children, there is evidence for the potential use of absolute CD4 cell count as an alternative to CD4 cell percentage in settings with limited resources. The trajectories for absolute CD4 count and CD4 cell percentage are similar over both 6-month and 24-month follow-up periods after ART initiation with similar slopes and trends. The changes from baseline for absolute CD4 count and CD4 cell percentage showed paralleled increases at each of the clinic visits.

There were differences in the use of absolute CD4 cell count and CD4 cell percentage demonstrated by this study that further support the potential use of absolute CD4 cell count as an acceptable alternative. Absolute CD4 cell count was found to be predictive of immunological treatment failures as well as significantly associated with WHO staging at baseline. CD4 cell percentage was neither a predictor variable for treatment failure nor associated with disease severity based on WHO staging. These findings may indicate an added value for absolute CD4 cell count as compared to CD4 cell percentage for both timing of ART initiation and monitoring of disease progression. For absolute CD4 cell count, there was no demonstrated difference between genders and an expected difference between age groups was observed.

The preliminary support for the use of absolute CD4 cell count evidenced by the study data has valuable public health implications due to technological advances in CD4 testing. There is already research supporting the economic incentives of absolute CD4 testing and this study supports clinical incentives for absolute CD4 testing. If point-of-care absolute CD4 cell count technology were acceptable in the pediatric HIV

community, the widespread implementation of this technology would have deep impact on the social and economic costs of monitoring pediatric HIV. Point-of-care technology, being developed only for absolute CD4 cell count capability, would allow ART monitoring to be done in a home-based program, which could limit stigma associated with HIV. In this study, guardians frequently expressed to research assistants their own feelings of being stigmatized themselves (if HIV-positive) and their concerns for any stigmatization of their children. These feelings and concerns are reflected by the low levels of disclosure about HIV diagnosis from guardians to children. Furthermore, it would reduce the costs associated with monitoring including school absence and transportation costs, which were significant concerns for participants in this study.

More analysis of the longitudinal CD4 data will be undertaken in future related research to examine differences in absolute CD4 cell count and CD4 cell percentage based on treatment effectiveness as well as associations with age, gender and other baseline variables. Mixed modeling will be used to analyze this longitudinal data. Data from this same cohort will continue to be collected and this author will be directly involved in the analysis and dissemination of this research.

C. Limitations

This study is relevant to the care and management of HIV-infected children in a resource-limited setting but does have limitations that should be considered when interpreting and applying the data. All the participants were recruited and enrolled from a single center and therefore caution must be used when generalizing the findings of this

study to other settings in Ghana. KBTH, being the largest hospital in the country, is not as limited in resources, manpower or facilities, compared to other medical facilities in Ghana. Therefore, applying the use of this data to settings in which a similar complement of care is not available may be limited. Furthermore, this study provided a small amount of funds to participants both as incentive and to defray the transportation costs. The patient costs to access care observed in this study may pose barriers to replicating study results as well as initiatives for universal access. Additionally, this study enrolled few children less than 5 years old and therefore is limited in ability to apply findings, particularly in the use of absolute CD4 cell count, to all age groups. This limitation may be a product of late HIV presentation and diagnosis at KBTH, further evidenced by the advanced age at ART initiation.

D. Conclusions

The development and advancement of ART have made a remarkable impact on morbidity and mortality attributable to pediatric HIV across the world. As the scale-up of available ART regimens progresses in the developing world, evaluation of the effectiveness of these available regimens is essential to ensure the success and safety of the scale-up process. Furthermore, low cost ways to monitor ART and disease progression are vital in conjunction with the scale-up of access to ART. This study demonstrates that the available ART regimens at Korle Bu Teaching Hospital in Accra, Ghana are effective, well tolerated and potentially durable for children with predominantly non-type B HIV infection. Results also show low rates of toxicity and

little evidence for disease resistance in ART-naïve participants. These results support the scale-up of universal access to the current ART regimens and encourage the production of low cost formulations to supply the need. However, study results emphasize the need for improvements in early HIV diagnosis and ART initiation. A shorter interval between diagnosis and treatment is a predictor of treatment failure of all types and may indicate poor outcomes for patients who initially present for care with advanced disease. Absolute CD4 cell count testing appears to have potential as an alternative to CD4 cell percentage for monitoring ART. The use of absolute CD4 cell count as a monitoring tool for HIV-infected children of all ages would allow for utilization of emerging, low-cost, point-of-care technologies with capability only for absolute CD4 count leading to potentially significant impact on the social and economic limitations of HIV disease management in resource-limited settings.

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