Pediatric HIV: Immunologic and Virologic Response to Antiretroviral Therapy

Kristjana Hrönn Ásbjörnsdóttir

A dissertation

submitted in partial fulfillment of the

requirements for the degree of

Doctor of Philosophy

University of Washington

2015

Reading Committee:

Grace John-Stewart, Chair

Jennifer Slyker

Stephen De Rosa

Program Authorized to Offer Degree:

Public Health - Epidemiology

University of Washington

Abstract

Pediatric HIV: Immunologic and Virologic Response to Antiretroviral Therapy

Kristjana Hrönn Ásbjörnsdóttir

Chair of the Supervisory Committee:
Professor Grace John-Stewart
Departments of Epidemiology, Global Health, Medicine and Pediatrics

Background: Virologic and immunologic responses to antiretroviral treatment (ART) in infants may differ from older children and adults due to immunologic, clinical or epidemiologic characteristics. There are few longitudinal studies of immune activation and changes in immune phenotype among infants.

Methods: Within a cohort of HIV-infected infants starting early ART in Nairobi, Kenya, longitudinal trajectories of HIV viral load and CD4 over two years of ART were modeled and compared to those of older children (Chapter 1). T-cell activation at baseline was measured using flow cytometry and its association with mortality and ART response quantified (Chapter 2). Changes in activation and T-cell memory subsets over two years of ART were described and compared among infants with good virologic and immunologic response to ART and those with discordant response (Chapter 3).

Results: Chapter 1: Infants had higher viral loads at baseline pre-ART, a slower rate of suppression and a higher post-ART stabilized viral load than children. Infants were less likely than children to suppress HIV viral load to <250 copies/ml following 6 months (32% vs. 73%, infants vs. children p<0.0001) and 2 years (75% vs. 89%, infants vs. children p=0.007) of ART. Children had a significantly faster rate of CD4% reconstitution than infants, but failed to catch up to infant CD4% (CD4% at 24 months: 29% vs. 22%, infants vs. children p=0.04).

Chapter 2: Among 75 infants, median CD8+ T-cell activation at baseline pre-ART was 16.5% (interquartile range [IQR] 9.9, 28.4). CD8+ T-cell activation was not correlated with pre-ART CD4%, HIV viral load or WHO stage. Low pre-ART CD8+ T-cell activation (<5%) was

associated with mortality overall (Hazard ratio (HR)=3.5 [95% CI 1.3, 9.4]); and among those who survived to start ART (HR 5.8, 95% CI). Pre-ART CD8+ T-cell activation >median was associated with slower CD4% recovery on ART.

Chapter 3: Among infants who completed two years of ART, different viral/immune patterns were noted – those who had viral suppression and CD4 recovery (responders), CD4 recovery despite lack of viral suppression (discordant responders), and those with neither good viral suppression nor immune recovery (nonresponders). Overall, CD8+ T-cell activation decreased substantially in the first six months on ART, and naïve CD8+ T-cells increased. Naive CD4+ T-cells increased among concordant good responders only.

Conclusion: Infants in this early ART cohort had high viral loads at baseline and failed to suppress virus or increase CD4% as efficiently as older children after ART initiation. In contrast to findings in adults, low CD8+ T-cell activation was associated with mortality and may be a marker of immune exhaustion and advanced HIV disease. Immune activation decreased and infant T-cell phenotype shifted toward a less differentiated phenotype with ART but was not restored to normal. Strategies to augment immune responses and improve ART specifically for infants are needed to improve outcomes in this vulnerable population.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGEMENTS

A great many people deserve thanks. My committee, who have been exceptionally generous with their time and insights; every meeting with each of you made me a better scientist. The study and clinic teams and the study participants and their families, without whom none of this work would be possible. Lab teams in Nairobi and the HVTN lab in Seattle, who made this logistically challenging project run as smoothly as it could, and who always made me feel welcome; the Predoc and Kizazi groups in the Kenya Research and Training Center for comments on the analysis. Many thanks to my parents, who started me on this path by moving us to Malawi when I was a child and who always entertained my questions about health and disease; special thanks for assisting in data collection for my first-ever paper on HIV when I was 10. And thanks to my husband - your support has been invaluable. Takk fyrir allt og allt.

Chapter 1. Differences in Virologic and Immunologic Response to Antiretroviral Therapy among HIV-Infected Infants and Children

1.1 BACKGROUND

Infant HIV viral loads are substantially greater than adult levels[1], and as many as 50% of untreated HIV infected African infants die by two years of age[2]. In contrast to acutely infected adults, vertically infected infants are slow to suppress virus[3] and many do not attain a discernable viral load set-point[4]. Older HIV infected children, the vast majority of whom represent vertically infected survivors, have lower viral loads than infants[5]. Due to high early mortality, untreated children who survive beyond 2 years of life likely have selected favorable viral or host characteristics[6].

Response to antiretroviral therapy may also differ between infants, children, and adults. In adults, ART typically results in up to 95% achieving viral suppression within 6 months of ART initiation, varying by the population included and the definition of suppression used[7-10]. Similar rates of viral suppression have been shown in older children[10,11]. However, infants have slower viral suppression[12,13] and higher incidence of viral failure[14]. Failure of ART viral suppression in infants may be due to higher baseline viral loads or challenges with ART adherence or dosing[15]. In addition, aspects of the immune response that cause poor viral control in untreated infants may contribute to slower viral decline with ART.

Immune recovery on ART usually occurs in tandem with viral decline[16,17]. In contrast to poor viral suppression with ART, infants on ART generally have better CD4 counts and percentages than older children[18-20]; this may be due to their high thymic output[21] and higher age-specific CD4 counts and CD4%[22-24], or to greater damage to the CD4 compartment among older children who have lived with untreated HIV for an extended period[25,26]. As ART response is primarily assessed using CD4 monitoring in developing country settings[27-30], treatment failure among infants in particular may be masked by their comparatively good immune status[27,30,31].

Although improved survival is achieved when ART is initiated early in infancy[32], in practice, infant diagnosis is often delayed[33] and early mortality on ART remains high[34]. There have been few longitudinal studies modeling rates of viral decline and immune recovery in early ART-treated infants and none comparing infants with older children. In order to understand whether impaired virologic and immunologic response to ART could explain high mortality post-ART in this population, we compared virologic and immunologic responses between infants starting ART in the first year of life versus children starting ART between the ages of 18 months to 12 years. Our hypotheses were that infants would have slower rates of viral suppression but comparable immune reconstitution to older children.

1.2 METHODS

1.2.1 *Study cohorts*

Data from two pediatric ART studies conducted in Nairobi, Kenya were used for these analyses. Recruitment and enrollment procedures have been described in detail elsewhere[34,35]. Both studies were approved by the University of Washington Institutional Review Board and the Kenyatta National Hospital Ethics and Research Committee. Each study has extended follow-up up to >5 years. The current study utilized data on immunologic and virologic markers obtained during the first 2 years of ART.

In the infant cohort [Optimizing Pediatric HAART (OPH) study, NCT00428116], HIV-infected infants less than 12 months old were identified at prevention of mother-to-child transmission of HIV (PMTCT) clinics and pediatric wards between 2007-2010. All infants were started on ART and followed monthly for growth, clinical outcomes and adherence for two years, at which time they were randomized to treatment interruption or continued treatment. HIV viral load was assessed at baseline and every three months throughout follow-up, while CD4 counts and CD4% were assessed every six months. For this analysis, only pre-randomization data were used.

In the child cohort [Pediatric Adherence (PAD) study, NCT00194545], children aged 18 months to 12 years with moderate to severe HIV disease (WHO disease stage II-IV) were enrolled from Kenyatta National Hospital HIV clinic and pediatric wards between 2004-2007. All children were started on ART and caregivers randomized to adherence counseling alone or adherence counseling with a medication diary, then followed monthly for growth, clinical indicators and self-reported adherence. HIV $\log_{10}$ viral loads were assessed every three months throughout follow-up, while CD4 counts and CD4% were assessed at the 0, 3, 6, 15, 21 and 27 month visits. For this analysis, data from the first two years post ART initiation were used.

1.2.2 *ART regimens*

Participants were treated according to contemporaneous Kenya national guidelines[28,36,37] and antiretrovirals were provided by the PEPFAR-supported youth HIV treatment program at the Kenyatta National Hospital Comprehensive Care Clinic (CCC). For the infant cohort, first-line treatment consisted of zidovudine (AZT), lamivudine (3TC) and either nevirapine (NVP) or ritonavir-boosted lopinavir for infants who were exposed to NVP for prevention of mother-to-child transmission (PMTCT). For the child cohort, first line treatment consisted of AZT and 3TC and either NVP or efavirenz.

1.2.3 *Laboratory Methods*

HIV viral loads were measured using the Gen Probe assay, which accurately quantifies the HIV-1 subtypes circulating in Kenya.[38] The lower limit of quantification for each study was determined by the volume of plasma tested; for the purposes of the current analysis, it was set as the higher of the values between cohorts, 250 copies/mL (2.39 $\log_{10}$ copies/mL). Viral load data were collected at the Fred Hutchinson Cancer Research Center in Seattle and were not available to clinicians during the studies. CD4 counts and CD4% were measured at Kenyatta

National Hospital throughout each study using flow cytometry and results were available to clinicians.

1.2.4 *Statistical Methods*

Unless otherwise noted, all analyses were conducted in Stata 14.0 (StataCorp, College Station, Texas). The two cohorts were compared for characteristics at baseline using Mann-Whitney U-tests for continuous variables and Chi square tests for categorical variables.

The probability of viral suppression at 3-monthly intervals among infants and children was estimated using life tables from Kaplan-Meier models; viral load suppression was defined as partial (HIV viral load <1000 copies/mL) and complete (HIV viral load<250 copies/mL). Time to first achievement of partial and full suppression post ART initiation was compared among infants and children using a log-rank test for survival functions. Time to first achievement of partial suppression by regimen (PI-containing vs. NNRTI-containing) among infants was compared using a long-rank test. In order to estimate the durability of viral suppression among infants compared to children, risk of viral rebound to >1000 copies/mL following full suppression was compared using Cox proportional hazards regression. This analysis was restricted to those who ever achieved full suppression, and time at risk began at the first fully suppressed visit.

The viral load trajectory in each cohort was estimated to follow the same pattern: high viral load pre-ART, followed by a period of exponential decline to a stabilized long-term cohort mean post-ART. The post-ART stabilized level reflects how completely viral load was suppressed in the cohort as a whole, while the slope reflects how rapidly the stabilized level was achieved. This pattern was modeled for each cohort using nonlinear mixed effects models, which were fitted using the *lme4* package in R (R Foundation for Statistical Computing, Vienna, Austria), and are described by the following formula:

Equation 1

$$VL(t) = VL_{post} + (VL_0 - VL_{post})e^{-rt}$$

where *VL(t)* is HIV $\log_{10}$ viral load at *t* days post-ART, VL_0 is HIV $\log_{10}$ viral load at baseline pre-ART, VL_{post} is the long-term population average stabilized HIV $\log_{10}$ viral load post ART and *r* is rate of decline. *r* may be interpreted the log of the proportion of suppression, $\log(VL(1) - VL_{post})/(VL_0 - VL_{post}))$, achieved in one time unit. Individual random effects are incorporated for VL_0 and VL_{post}. 95% confidence intervals for mean model estimates were simulated using bootstrap with 1000 repetitions and the bootstrap distributions were compared using the Kolmogorov-Smirnov test.

CD4% reconstitution on ART was estimated to occur in two phases: a sharp increase from baseline to the 6 month measurement (days on ART≤210 to account for late visits) followed by a more gradual continued increase from the 6 month visit to the end of follow-up at two years (days on ART≤750). The difference in slope between the infant and older child cohorts in each phase was assessed using interaction terms. CD4% at each time-point post-ART is modeled by the following formula:

Equation 2

$$log(CD4\%_{ij} = log(CD4\%_6) + \beta_1 I + \beta_2 T_{1ARTij} + \beta_3 T_{2ART} + \beta_4 I_j * T_{1ARTij} + \beta_5 I_j * T_{2ARTij} + \epsilon_{ij}$$

where $CD4\%_{ij}$ is the CD4% for subject *j* at a given time *i* post-ART; $CD4\%_{6i}$ is the CD4% for subject *j* at 210 days, T_{1ART} is the number of days before 210 days (T_{1ART}=210 at ART initiation) and T_{2ART} is the number of days after 210 days, *I* indicates whether subject *j* is an infant, and ϵ_{ij} is an error term. Individual random effects were incorporated for intercepts and slopes, using an exchangeable correlation structure. CD4% was log-transformed to improve normality of the data.

CD4 counts and CD4% in healthy children are highest in infancy and decline with age, so that age-specific expected or "healthy" CD4 counts and percentages vary throughout childhood. As such, infants are more immunocompromised at a given CD4% than older children. To correct for this difference, a published model of CD4 count decline with age in HIV-uninfected children[39] was used to predict age-specific expected CD4 counts at each time-point

and calculate the ratio of each child's observed CD4 count to their age-specific expected CD4 count; the ratio is hereafter called "CD4-for-age". CD4-for-age equal to 1 means a normal CD4 count has been achieved. The increase in CD4-for-age over time on ART was compared between the two cohorts. Reconstitution to stabilized CD4-for-age on ART has been described in pediatric ART cohorts previously[18,20]; however, the asymptotic model used in these studies was a poor fit for our data, likely because two years of follow-up are not sufficient to achieve a long-term stabilized CD4-for-age. Instead, we modeled log-transformed CD4-for-age using a model similar to the linear mixed effects model in Equation 2.

We conducted sensitivity analyses to assess the impact of survival bias and adherence on our analyses. To directly compare treatment response among acutely-infected infants and chronically infected children who had survived the highest-risk period without ART, sensitivity analyses were conducted excluding infants over six months old and children under two years old. In order to remove the effect of consistent non-adherence on estimates of rate of CD4% reconstitution and viral load suppression, sensitivity analyses were conducted excluding participants who never achieved viral load suppression in the first two years on ART.

1.3 RESULTS

1.3.1 *Study population characteristics at enrollment*

Among 121 infants in the infant cohort, the median age at enrollment was 3.9 months (IQR 3.3-5.0) (Table 1.1). Among 124 children in the child cohort, median age at enrollment was 58 months (IQR 32-76), or 4.8 years. Among infants, 38% started a protease-inhibitor (PI)-based regimen as first-line treatment, whereas none of the older children did. Older children had more advanced disease at ART initiation; 88% met WHO criteria for stage III or IV compared to 30% of infants ($p<0.0001$). Nutritional status at baseline was poor in both cohorts, with median weight-for-age Z-score (WAZ) -2.4 among infants and -2.3 among older children. In both

cohorts, most children were cared for by biological parents, although 26% of older children had lost their biological mothers compared to just 2.5% of infants (p<0.0001).

1.3.2 *Viral suppression*

During the two years following ART, a greater proportion of older children than infants ever achieved partial (<1000 copies/mL, Figure 1.1A) and full (<250 copies/mL, Figure 1.1B) HIV viral load suppression. Time to partial suppression was longer among infants than children (median 159 days among infants vs. 91 days among children, p=0.0075), as was time to full suppression (median 265 days among infants vs. 98 days among children, p<0.0001). Following 6 months of ART, 73% of children and 32% of infants fully suppressed virus (p<0.0001). By 24 months on ART, 93% of children and 81% of infants had ever suppressed to <1000 copies/mL (p=0.009) and 89% of children and 75% of infants had suppressed to <250 copies/mL (p=0.007). Infants were at increased risk of viral rebound to >1000 copies/mL following full suppression (Hazard Ratio (HR)=2.1 (95% CI 1.2-3.5), p=0.005). There was no difference in time to partial suppression among infants by PI vs. NNRTI regimen (p=0.37).

From the nonlinear mixed effects model, estimated mean infant viral load (VL) prior to ART was >0.5 $\log_{10}$ higher than the pre-ART VL in the child cohort (6.47 versus 5.91 $\log_{10}$ copies/ml in infants vs. children, Figure 1.2A, Table 1.2). Population-stabilized viral load post-ART (VL_{post}) was higher among infants than children (3.49 $\log_{10}$ vs. 2.86 $\log_{10}$ copies/mL, p<0.001). The rate of decline parameter *r* was larger in older children than infants (p<0.001), such that among infants viral load declined 50% of the way toward the population-stabilized post treatment viral load in 17 days, and 90% in 55 days; stabilized post-ART viral load was reached at >110 days (Figure 1.2B). In the child cohort, viral load declined 50% of the way toward stabilized viral load in 11 days and 90% of the way in 36 days; stabilized post-ART viral load was reached at >71 days. In sensitivity analyses, exclusion of infants greater than six months of age and children less than 24 months of age did not substantially alter these estimates (Table

1.2). When analysis was limited to children who ever achieved full suppression, rate of viral suppression remained slower in the infant cohort (Table 1.2, Figure 1.2C).

1.3.3 *CD4% reconstitution*

Children were substantially more immunosuppressed than infants at baseline pre-ART (geometric mean CD4 7.3% vs. 19.0%, p<0.001, Figure 1.3A). Infant CD4% remained higher throughout follow-up, due to the large difference in baseline immune status. Over two years of ART, 50% of children and 90% of infants ever reconstituted CD4 to 25% (data not shown). From the linear mixed effects model, children had more rapid CD4 reconstitution throughout follow-up than infants (Table 1.3). For an average child, CD4% increased 1.45-fold per 100 days in the first 6 months on ART, compared to a 1.15-fold increase per 100 days in the first 6 months for an average infant. By 6 months, geometric mean CD4 was 15.7% for an average child and 25.4% for an average infant. In the second phase, both cohorts had a slower rate of increase, and only the child slope remained statistically significant (1.07-fold increase/100 days for an average child vs. 1.02-fold increase/100 days for an average infant). The difference in slopes was strongly significant in the first phase (p<0.001) but not significant in the second phase (p=0.11). After two years of ART, the difference in CD4% between cohorts had decreased but geometric mean CD4% remained lower in the child than infant cohort (22% vs. 29%, p=0.04). When infants over 6 months old and children under two years old were excluded, as in the main analysis, infants had higher CD4% throughout follow-up, with the difference in CD4% between cohorts slightly greater and the infant CD4% reconstitution slightly more rapid. When the analysis was limited to those who ever achieved full viral suppression, the point estimates were not statistically significantly different from those in the main analysis, but differences between infants and children persisted.

1.3.4 *CD4-for-age reconstitution*

When the rate of increase of CD4-for-age was modeled, infants had significantly higher pre-ART levels than children (geometric mean 0.41 vs. 0.19 CD4-for-age, p<0.001, Figure 3B). Older children showed more rapid reconstitution in both the first phase (2.4-fold vs. 1.6-fold increase / 6 months, p<0.001) and the second phase (1.19-fold vs. 1.07-fold increase / 6 months, p=0.001), and the difference was statistically significant in both phases. Despite this, over two years of ART, infants had consistently higher CD4-for-age; 24-month values were 0.70 among children compared to 0.77 among infants (p=0.04).

1.4 DISCUSSION

Response to ART differed substantially among infants and children in our study. Despite their less advanced clinical disease and greater age-adjusted CD4 at baseline, infants had higher viral loads and substantially worse virological response to ART than older children. In contrast, older children failed to catch up to infant CD4% or CD4-for-age following two years of ART.

We found that the rate of viral suppression was markedly slower in infants than in children. Fewer infants than children achieved viral suppression at any time during follow-up, and infants who fully suppressed virus were at higher risk of viral rebound after initial suppression. The population-mean stabilized viral load post-ART was also higher in infants than children, reflecting the continued prevalence of detectable virus in the infant cohort at all time points throughout follow-up; this was due to both the smaller proportion of infants ever achieving full suppression and the larger proportion with viral rebound after initial suppression. Virologic failure in pediatric populations occurs frequently[14,40-42] and is of particular concern in developing countries, owing to the limited availability of second-line treatment regimens.

Various factors may explain poor viral suppression in infants. Ideal antiretroviral dosage and adherence may be particularly challenging in pediatric populations[15,43,44] and syrup dosing

may be hard for caregivers to calibrate,[45,46] particularly among infants due to frequent regurgitation or emesis. The relative ease of administering medication to older children compared to infants may partly explain the differences we observed. To exclude differential non-adherence as a cause of differing rates of viral suppression among infants and children, we conducted a sensitivity analysis restricted to those who achieved viral suppression. In this analysis, rates of viral load suppression remained slower among infants, suggesting that adherence alone did not explain different rates of viral suppression.

Untreated infants have less viral control than older children and adults[4], and the mediators of poor viral control in the absence of ART may contribute to poor viral suppression with ART in infants. Higher baseline viral levels in infants would not necessarily lead to slower rate of viral decline but could have contributed to longer time to suppression[47]. It is unclear why infants control virus more poorly than adults in the absence of treatment. It is possible that higher infant CD4 counts[39] contribute more target cells for viral infection and replication; alternatively, an immature immune response may explain their poor viral control. Infants share maternal HLA-types and may be infected with viruses that escaped maternal HLA-restricted immune responses[48]. Infants infected later postpartum have lower viral loads and mortality than those infected before the first month of life[1], either due to a lower infective dose or more effective immune responses. It is possible that infants' immature immune systems were less able to synergistically control virus concurrent with ART, and that older children, being a select group of survivors, represented those who were able to mount a more effective immune response in infancy. Complementary strategies such as therapeutic vaccines or immunotherapies may be useful to accelerate suppression in this critical period.

Compared to infants, children had much more rapid rates of CD4% recovery in the first six months and more rapid rates of CD4-for-age increase throughout the two years of follow-up. This difference between our CD4% and CD4-for-age models highlights the importance of taking into account age-specific healthy levels when estimating immune reconstitution in pediatric

populations. Despite their greater rate of recovery, children failed to catch up to infant CD4% and CD4-for-age following two years of ART. This is consistent with previous studies.[18,19,49,50] Similar to our CD4-for-age model, a recent study from Uganda and Zimbabwe found lower CD4-for-age at ART initiation was associated with both older age at ART initiation and more rapid initial slope in CD4-for-age reconstitution[20]; long-term predicted CD4 count was higher among children who initiated ART at a younger age in that study[20]. While some studies have suggested that most children can restore normal CD4 with treatment[51], others have concluded that those who initiate ART at an older age[18,20] or when more immunocompromised[25] are unlikely ever to normalize CD4 count. Immune recovery despite lack of viral suppression has been described in many pediatric cohorts[16,17,52,53]. In these studies, viral responders generally reconstitute CD4 more rapidly than non-responders[17,53]. Similarly, we saw a trend toward greater CD4% increase among those with viral suppression in our study, although the difference in rate parameters was not significant.

Our study had several strengths and limitations. Mixed effects models took advantage of longitudinal data available in both cohorts to describe immunologic and virologic response in detail. We conducted several sensitivity analyses, and were able to establish that differences between infants and children persisted when restricted to those who achieved viral suppression, and were slightly more pronounced when infants under six months and children older than two years were compared. Limitations include some regimen differences between cohorts, with some infants but no children receiving PI-containing first-line regimens. However, differences in regimen were not associated with time to viral suppression in the infant cohort. Exclusion of children at WHO stage I from the child study was also a limitation; however, as older children are often symptomatic[54] and have low CD4%[55,56] at diagnosis, the study population is fairly representative of children presenting to care in developing country settings.

In our cohort, despite ART initiation by a median of four months of age, viral suppression was slow among infants and many failed to suppress throughout two years of ART. Combined

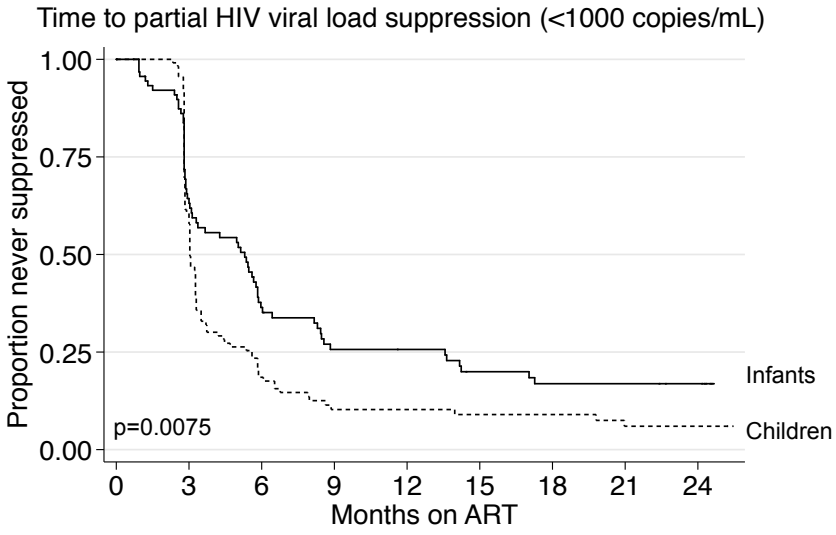
with the high early mortality among infants on ART[34], our results suggest that improvement of early ART regimens to achieve rates of suppression more comparable to those in older populations could improve prognosis and reduce stabilized viral loads early in infection. New formulations specifically for infants are needed to address dosage and adherence challenges; complementary immune strategies also may enhance early ART responses in HIV-infected infants. Monitoring of CD4% is insufficient to identify children at risk of viral failure, and regular viral load monitoring is particularly important in younger populations among whom CD4 counts may appear healthy even in the presence of very high viral loads. Where viral loads are unavailable, CD4-for-age may be a more sensitive proxy measure than CD4%.

Table 1.1 Baseline characteristics of infants and older children who started ART.

	Infant cohort N=121 n (%) or median (IQR)	Child cohort N=124 n (%) or median (IQR)	p-value
Child characteristics			
Age at enrollment (months)	3.9 (3.3, 5,0)	58 (32, 76)	—
First line treatment			
NRTI/NNRTI	75 (62)	124 (100)	—
NRTI/PI	46 (38)	0 (0)	
Female	61 (50)	64 (52)	0.49
WHO disease stage			<0.0001
I/II	69 (71)	12 (9.8)	
III	31 (27)	94 (77)	
IV	4 (3.4)	16 (13)	
CD4 count (cells/μL)	1400 (800, 2100)	280 (96, 550)	<0.0001
CD4%	21 (16, 29)	6.3 (3.2,11)	<0.0001
Log_{10} HIV viral load	6.6 (6.1, 7.0)	6.0 (5.4, 6.5)	<0.0001
Height-for-age Z-score	-2.1 (-3.2, -0.97)	-2.4 (-3.4, -1.3)	0.32
Weight-for-age Z-score	-2.4 (-3.8, -1.0)	-2.3 (-3.1, -1.4)	0.31
Weight-for-length Z-score	-1.0 (-2.4, -0.06)	-1.1 (-2.0, -0.20)	0.64
Caregiver characteristics			
Biological mother alive	118 (98)	92 (74)	<0.0001
Mother received ART for PMTCT[1]	50 (45)	N/A	—
Parental history of ART for own health[2]	20 (16)	11 (13)	0.28
More than 8 years of education	50 (41)	115 (74)	<0.0001
One room house	70 (57)	70 (57)	>0.99

[1] Information on PMTCT was not available in the child cohort.
[2] In the infant cohort, maternal history of ART was collected; in the child cohort, the question referred to either parent.

A



B

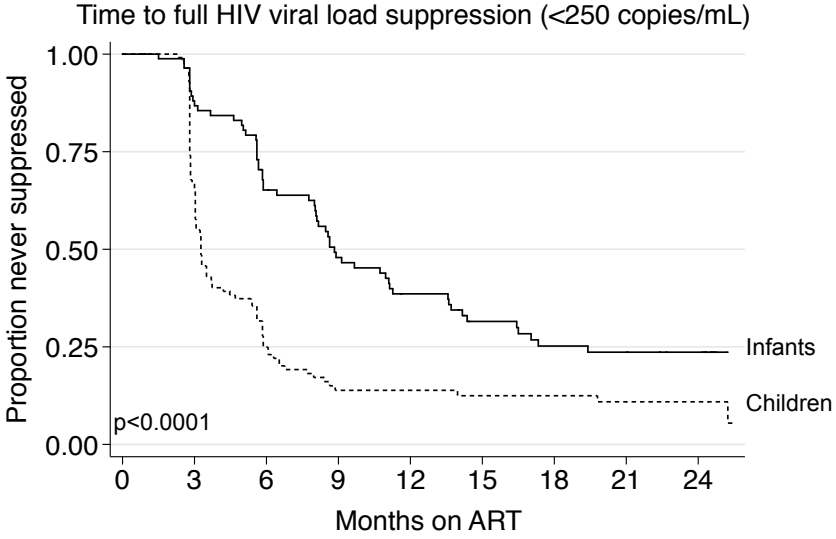


Figure 1.1 Time to viral suppression. Time to first partial (<1000 copies/mL, Panel A) and full (<250 copies/mL, Panel B) HIV viral load suppression after ART initiation in the infant and child cohorts. P-values are from the log-rank test for equality of survival functions.

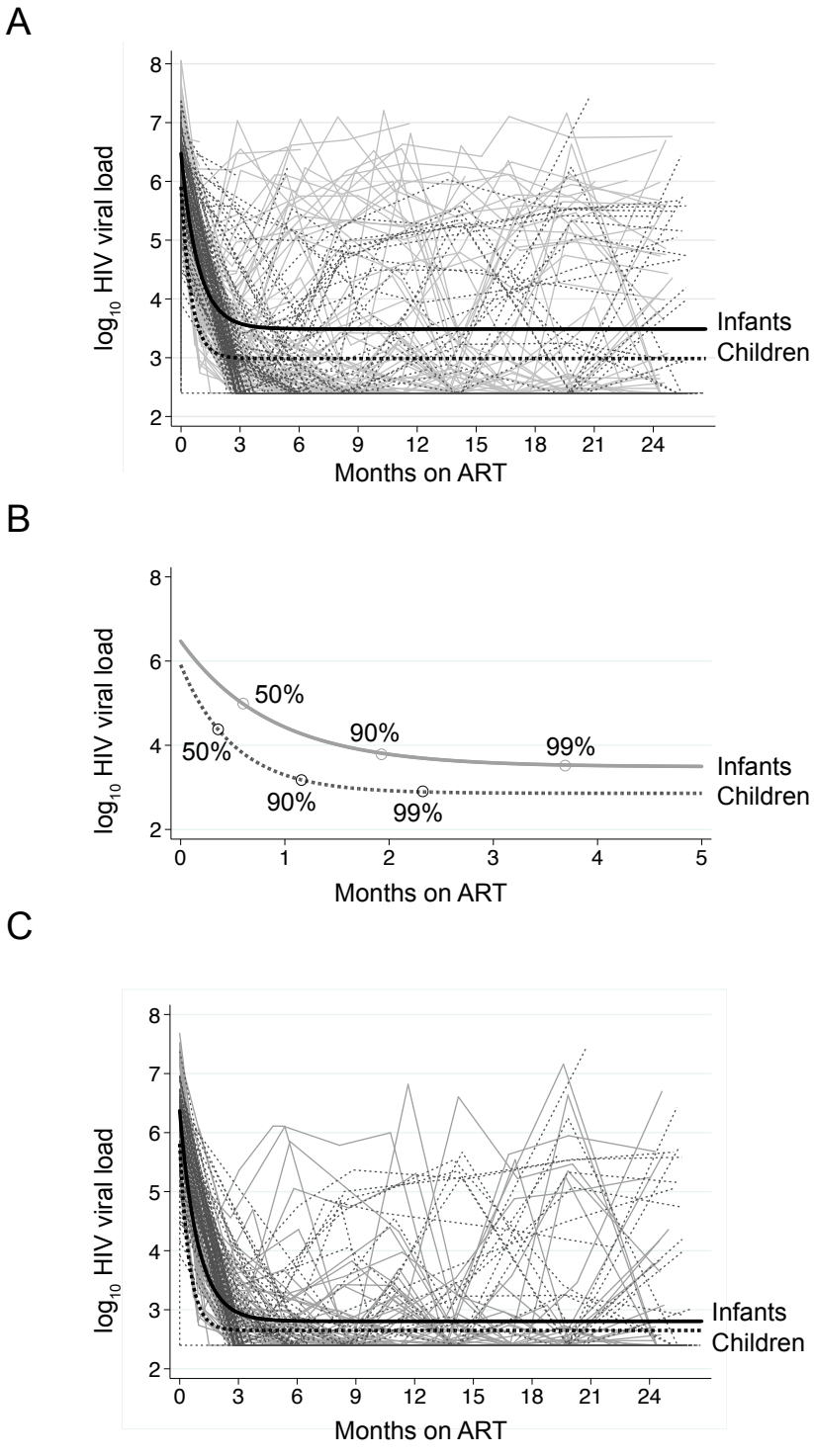
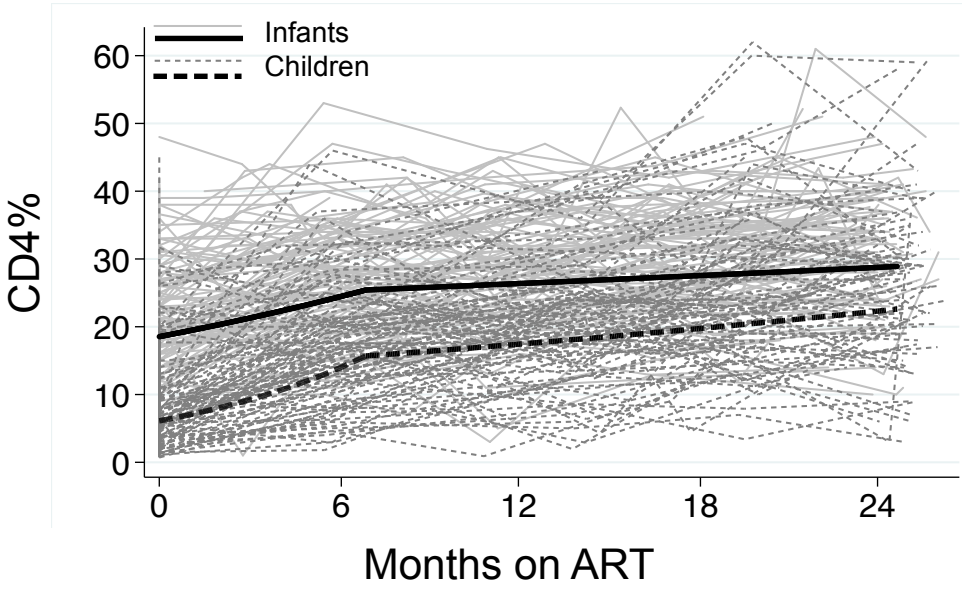


Figure 1.2 HIV viral load trajectories on ART in the infant and child cohorts. Panel A shows Individual trajectories of $\log_{10}$ HIV viral load in the infant (solid) and child (dashed) cohorts over two years of follow-up, overlaid with the population mean for each cohort. Panel B shows the population mean $\log_{10}$ HIV viral load in each cohort over time, with the time to 50%, 90% and 99% of stabilized level labeled. Panel C shows individual trajectories of $\log_{10}$ HIV viral load in the infant and child cohorts, restricted to those who achieved full suppression (<250 copies/mL) at one or more visits, overlaid with the population mean for each cohort.

Table 1.2 Mean rate of HIV viral load suppression on ART among infants and children. Estimates are from nonlinear mixed effects models fit to each cohort's data; p-values are obtained by comparing distributions of estimates from 1000 bootstrap repetitions..

	VL_0 (95% CI)	VL_{post} (95% CI)	r (95% CI)	Days to % VL_0-VL_{post} 50	75	90	99
Child	5.91 (5.79, 6.05)	2.86 (2.66, 2.92)	-2.7 (-3.3, -2.5)	11	21	36	71
Infant	6.47 (6.37, 6.64)	3.49 (3.15, 3.66)	-3.2 (-3.3, -2.8)	17	33	55	110
p-value	*<0.001*	*<0.001*	*<0.001*				
Excluding infants >6 months old and children < 2 years old							
Child	5.83 (5.67, 5.99)	2.82 (2.64, 2.90)	-2.9 (-3.4, -2.5)	13	26	43	86
Infant	6.51 (6.38, 6.71)	3.53 (3.17, 3.78)	-3.2 (-3.4, -2.8)	18	35	59	118
p-value	*<0.001*	*<0.001*	*<0.001*				
Excluding children who never suppressed HIV viral load							
Child	5.81 (5.64, 6.00)	2.65 (2.54, 2.75)	-2.7 (-3.1, -2.5)	10	20	34	68
Infant	6.37 (6.20, 6.56)	2.81 (2.69, 2.97)	-3.3 (-3.4, -3.2)	19	39	64	129
p-value	*<0.001*	*<0.001*	*<0.001*				

A



B

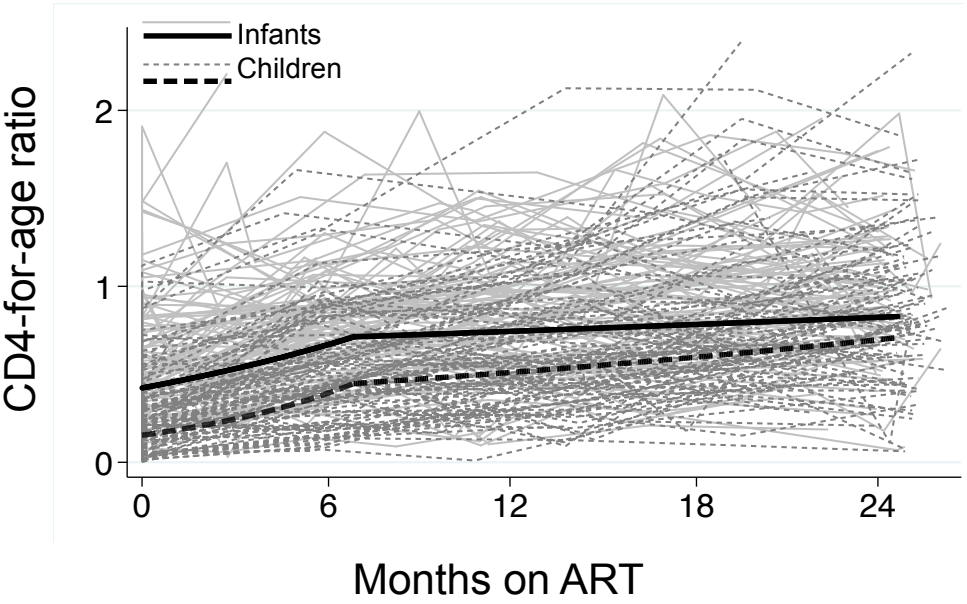


Figure 1.3 CD4 trajectories on ART in the infant and child cohorts. Panel A shows individual CD4% trajectories in the infant and child cohorts. Black lines show the mean CD4% trajectories in each cohort, estimated using a linear mixed effects model. Panel B shows individual trajectories of CD4-for-age, overlaid with the mean CD4-for-age trajectories for each cohort estimated using a linear mixed effects model.

Table 1.3 Mean rate of CD4% reconstitution on ART among infants vs. children, estimated using a linear mixed effects model. The model contained two slopes, one from baseline to the 6 month measurement (days on ART≤210 to account for late visits) and the other from the 6 month visit to the end of follow-up at two years (days on ART≤750).

	Child	(95% CI)	Infant	(95% CI)	p-value
Including all children					
log(CD4%) at six months	2.75	(2.67, 2.84)	3.24	(3.16, 3.31)	<0.001
CD4% at six months	*15.7*	*(14.4, 17.1)*	*25.4*	*(23.6, 27.4)*	
First six months slope (/100 days)	0.45	(0.39, 0.51)	0.15	(0.10, 0.20)	<0.001
Six months - 2 years slope (/100 days)	0.07	(0.03, 0.11)	0.02	(-0.02, 0.06)	0.11
Excluding infants >6 months old and children < 2 years old					
log(CD4%) at six months	2.75	(2.66, 2.85)	3.25	(3.14, 3.36)	<0.001
CD4% at six months	*15.7*	*(14.3, 17.3)*	*25.8*	*(23.1, 28.8)*	
First six months slope (/100 days)	0.48	(0.41, 0.54)	0.20	(0.13, 0.28)	<0.001
Six months - 2 years slope (/100 days)	0.06	(0.01, 0.10)	0.02	(-0.04, 0.08)	0.33
Excluding children who never suppressed HIV viral load					
log(CD4%) at 6 months	2.78	(2.70, 2.87)	3.33	(3.23, 3.42)	<0.001
CD4% at six months	*16.1*	*(14.9, 17.6)*	*27.8*	*(25.3, 30.6)*	
First six months slope (/100 days)	0.43	(0.36-0.49)	0.17	(0.09, 0.25)	<0.001
Six months - 2 years slope (/100 days)	0.07	(0.03-0.11)	0.02	(-0.03, 0.07)	0.13

Chapter 2. CD8+ T-cell activation predicts mortality and treatment outcomes among infants initiating early ART

2.1 BACKGROUND

Immune activation is a critical step in the immune response to infection; however, chronic immune activation in HIV infection[57] has been associated with poor outcomes in both the presence and absence of antiretroviral therapy (ART). In untreated adult cohorts, T-cell activation (%CD38+/HLA-DR+) is a strong predictor of rate of progression to AIDS[58-61] and death[62]. In pediatric cohorts, HIV infection is associated with increased immune activation[63-65] and increased CD8+ T-cell activation has been observed as early as two weeks of age among perinatally infected infants[66]. T-cell activation among children declines following initiation of ART[49,67], returning to levels observed in HIV-uninfected children in some[68] but not all studies.[69,70] In ART-treated children, residual immune activation remains associated with viral load,[67,70] CD4%,[67] and time to viral failure.[71]

Few pediatric studies have assessed the association between pre-treatment immune activation and ART response. Among children switching from zidovudine monotherapy to highly active antiretroviral treatment (HAART) in a US study, CD8+ T-cell activation was associated with persistent detectable viral load.[72] However, immune activation did not predict virologic response to ART in an Italian study[17]. There are limited data in infants, a particularly high-risk group with two-year mortality as high as 50% in the absence of treatment[2] and who generally demonstrate slower viral suppression following ART than adults.[12,34,73] Differences in infant immune activation may partly explain the particularly poor prognosis of infants.

We hypothesized that high T-cell activation would be associated with increased disease severity, poor response to ART, and increased risk of mortality among infants. We determined levels and correlates of T-cell activation in ART-naive HIV infected infants and quantified the association between baseline immune activation, survival and ART treatment response.

2.2 METHODS

2.2.1 *Study cohort*

This study was approved by the Institutional Review Board of the University of Washington and by the Kenyatta National Hospital Ethics and Research Committee. The Optimizing Pediatric HAART study (NCT00428116) recruitment and enrollment procedures have been described in detail elsewhere[34]. HIV-infected infants less than 5 months of age were enrolled from prevention of mother-to-child transmission (PMTCT) clinics and pediatric hospital wards in Nairobi, Kenya immediately after their HIV diagnosis, between 2007-2010. Infants' caregivers provided written informed consent for participation. Infants were started on ART and followed monthly for two years, at which point those with CD4>25% and adequate growth were randomized to treatment interruption or continued treatment. The current analysis uses data from the pre-randomization period. Growth and clinical information were collected monthly, HIV $\log_{10}$ viral load was measured every 3 months and CD4 count and CD4% every 6 months. Infant blood was drawn at baseline pre-ART and every three months throughout follow-up and peripheral blood mononuclear cells (PBMCs) isolated and cryopreserved. All infants with an available baseline PBMC sample were included in these analyses.

2.2.2 *Laboratory methods*

T-cell phenotypes were analyzed by flow cytometry, according to a published protocol[74]. Briefly, cryopreserved PBMCs from infant blood samples were thawed into R10 buffer (RPMI 1640 with 25mM HEPES buffer and L-glutamine plus 10% FBS, 1% L-glutamine and 1% Penicillin-Streptomycin; Gibco BRL Life Technologies). PBMCs were stained with AViD dead cell stain (Life Technologies), then with a cocktail of T-cell phenotyping surface antibodies (Table 2.4 Supplementary). All antibodies were titrated to appropriate concentrations before being used in the assay. Cells were permeabilized with FACS Lyse / FACS Perm II (BD

Biosciences) and finally stained with intracellular antibodies. Stained cells were resuspended in 1% Paraformaldehyde (Electron Microscopy Sciences) solution for collection on an LSR II instrument (BD Biosciences).

Samples collected on the LSR II were analyzed using FlowJo 9.7.6 (Tree Star, Ashland, Oregon). The T lymphocyte population (CD3+) was separated into CD4+ T-cells (CD4+CD8-) and CD8+ T-cells (CD4-CD8+). Three activation-related phenotypic marker combinations were assessed, and the proportion $CD38^{high}$/HLA-DR+ ("activated"), Ki67+/$Bcl2^{low}$ ("cycling") and CD95+/$Bcl2^{low}$ ("apoptosis-vulnerable") quantified separately within CD4+ and CD8+ T-cell populations (Table 2.2; Figure 2.1). Data on memory subsets within the CD4+ and CD8+ T-cell populations were collected but are not discussed in this report.

2.2.3 *Statistical methods*

Statistical analyses were performed using Stata 14.0 (StataCorp, College Station, Texas). Associations between infant characteristics at baseline and levels of CD4+ and CD8+ T-cell activation were assessed using Mann-Whitney U-tests for dichotomous variables and Spearman's rank correlation for continuous variables. Correlation between immune parameters was assessed using Spearman's rank correlation.

For longitudinal analyses, CD8+ T-cell activation was dichotomized at two thresholds defined *a priori:* greater or less than median and greater or less than 5%.

The association between baseline activation and partial (<1000 copies/mL) and complete (<150 copies/mL) HIV viral load suppression was assessed using Cox proportional hazards regression, and effect modification by ART regimen tested using an interaction term. The association between baseline CD8+ T-cell activation and mortality, overall and among the subset of infants who initiated ART, was also assessed using Cox proportional hazards regression, with confounding by WHO disease stage and $\log_{10}$ HIV viral load assessed in

separate models. The proportional hazards assumption was tested in each model by inclusion of activation as a time-varying covariate.

The association between CD8+ T-cell activation at baseline and CD4% reconstitution over the first two years of ART was assessed using a linear mixed effects model. The rate of reconstitution was modeled using one slope for initial CD4% reconstitution between baseline and 6 months and another for the slower continued recovery between 6 and 24 months. Individual variation by infant was incorporated using random intercepts and slopes and an exchangeable correlation matrix.

2.3 RESULTS

2.3.1 *Study population*

Among 88 infants with baseline specimens collected pre-ART, 75 had PBMCs available for flow cytometry studies; the median age of these infants was 4 months (interquartile range (IQR) 3.3-4.6, Table 2.1). The median viral load prior to ART was 6.6 $\log_{10}$ copies/mL (IQR 6.1-7.2). More than half the infants presented with advanced disease, with 52% meeting criteria for WHO disease stage III/IV and 67% hospitalized at the time of their HIV diagnosis and enrollment into the study. Less than half the infants (41%) had been exposed to PMTCT. During the study, 59 (79%) infants initiated ART, of whom 41% received a nevirapine-based regimen as first-line treatment and 59% a lopinavir/ritonavir- based regimen. The remaining 16 infants died (n=12) or were lost to follow-up (n=4) without starting ART.

2.3.2 *CD4+ T cell activation*

At baseline, pre-ART, a median of 3.3% of CD4 T+ cells were activated (IQR 1.6-5.5%, Table 2.2), 4.5% cycling (IQR 2.2-9.8%), and 15.8% were apoptosis-vulnerable (IQR 9.2-28.9%). There was a moderate correlation between the proportions activated and cycling

(ρ=0.49, p<0.0001) and a stronger correlation between the proportions activated and apoptosis-vulnerable (ρ=0.60, p<0.0001).

2.3.3 *CD8+ T-cell activation*

A larger proportion of cells in the CD8+ subset were activated compared to the CD4+ subset. A median of 16.5% of CD8+ T cells were activated (IQR 9.9-28.4%, Table 2a), 12.8% were cycling (IQR 7.3-23.9%), and 13.1% were apoptosis-vulnerable (IQR 6.8-21.6%). In the CD8+ T cell subset, the percentage of activated cells was strongly correlated with the percentage of cycling cells (ρ=0.78, p<0.0001), and the percentage of apoptosis-vulnerable cells (ρ=0.70, p<0.0001). The proportions of CD4+ and CD8+ T-cells activated were moderately correlated (ρ=0.49, p<0.0001).

2.3.4 *Correlates of T cell activation*

There was a weak, but significant, inverse correlation between CD4+ T-cell activation and CD4% (ρ=-0.263, p=0.03, Table 2.3); but no correlation between CD4+ T-cell activation and HIV viral load, WHO disease stage, age, or growth score.

CD8+ T-cell activation at baseline was highly variable (range 0.3-60%) and was not correlated with HIV viral load, CD4% or WHO disease stage (Table 2.3, Figure 2.2). There was a trend for less CD8+ T cell activation in older infants (ρ=-0.211, p=0.07), and a trend for higher activation among infants with better weight-for-age (ρ=0.205, p=0.08) and length-for-age Z-scores (ρ=0.198, p=0.09).

2.3.5 *Mortality*

Of 75 infants, 24 died at a median of 25 days post study entry (IQR 9-63). Half of these (12/24) died before ART was initiated, at a median of 11 days post study entry (IQR 5-27). All the remaining deaths occurred within 6 months of ART initiation, at a median of 50 days post-ART, with the last occurring at 185 days post-ART.

CD4+ T cell activation was not associated with mortality in any analyses. CD8+ T-cell activation above the cohort median was not associated with the risk of mortality in a univariate analysis (Hazard Ratio (HR)=0.62, 95%CI 0.27-1.42, p=0.26; Figure 2.3A). However, CD8+ T-cell activation at levels comparable to healthy HIV-negative individuals (<5% CD8+CD38^{high}/HLA-DR+) was associated with more than a 3-fold increased risk of mortality among all infants (HR=3.5 (95% CI 1.3-9.4), p=0.01; Figure 2.3B) and more than 5-fold increased risk among those who initiated ART (HR=5.3, 95% CI 1.4-19.7, p=0.01; Figure 2.3C). When CD8+ T-cell activation was divided into three categories (<5%, ≥5% and < median, ≥median, Figure 2.3D), there was no difference in survival between those in the ≥5% to median and ≥median categories (p=0.62) but significant differences persisted between infants in the <5% category and each of the others (p=0.03 for both comparisons). All results were comparable when controlling for HIV viral load (data not shown). After adjusting for WHO disease stage, infants with <5% CD8+ T-cell activation remained at increased risk of mortality overall (adjusted HR (aHR)=3.0, 95%CI 1.1-8.1, p=0.03) and post-ART (aHR=4.8, 95%CI 1.3-18.0, p=0.02).

Of seven infants with <5% CD8+ T-cell activation at baseline, two died prior to ART initiation and three died while on ART. Five of the 7 infants were WHO stage III or IV (Table 2.5 Supplementary) and 4 had $\log_{10}$ HIV viral loads greater than 7.0. All but one were severely underweight (WAZ<-3.0) and 5 were severely stunted (LAZ<-3). Six of 7 infants were hospitalized at the time of enrollment.

2.3.6 *CD4% recovery*

Fifty-nine infants started ART and were included in analyses of CD4% recovery. Mean CD4% at baseline pre-ART among these infants was 19.9%. CD4% increased rapidly during the first 6 months of ART in the cohort overall (1.2%/30 days, p<0.001) followed by a slower increase from 6-24 months (0.26%/30 days, p<0.001). CD8+ T-cell activation >median at

baseline was associated with significantly slower CD4% reconstitution during the first 6 months (0.77%/30 days vs. 1.74%/30 days, p=0.01; Figure 2.4A) but not between 6 and 24 months (0.31%/30 days vs. 0.19%/30 days, p=0.83).

2.3.7 *Viral suppression*

Fifty-six infants had at least one viral load measurement post-ART and were included in the viral suppression analysis. Viral suppression was slow in the cohort; 80% of infants achieved partial HIV viral load suppression (<1000 copies/mL) at any time during the first two years on ART, and 46% achieved full suppression to undetectable levels (<150 copies/mL). There was no association between CD8+ T-cell activation >median and partial HIV viral load suppression (<1000 copies/mL; HR=0.95, 95%CI 0.48-1.86, p=0.88; Figure 2.4B) or full HIV viral load suppression (<150 copies/mL; HR=1.35, 95%CI 0.65-2.79, p=0.42; Figure 2.4C). There was no effect modification by ART regimen observed (data not shown).

2.4 DISCUSSION

In this cohort of HIV-infected infants, the percentage of activated T-cells at baseline before ART initiation was highly variable and was not associated with HIV viral load or WHO disease stage. Contrary to our hypothesis, infants with CD8+ T-cell activation percentages in the range of HIV-uninfected individuals (<5%) had a higher risk of mortality, and this association was independent of WHO disease stage and HIV viral load. Among infants who survived and had follow-up CD4% data on ART, high CD8+ T-cell activation was associated with slower immune reconstitution on ART.

We did not find an association of baseline pre-ART CD8+ T-cell activation with viral load or WHO stage in our cohort. This is consistent with the results of two large studies of Ugandan children aged 1-11 years[68,75] and a smaller US study of rapid vs. non-rapid progression of a similar age range[76], none of which found an association between HIV viral load and activation.

In contrast, a cross-sectional study from South Africa of children aged 4 months-11 years found a correlation between CD8+CD38+ T-cell count and HIV $\log_{10}$ viral load[73]. Immune activation in children with HIV may be driven by a variety of antigens other than HIV itself, including coinfections[77] and bacterial products in the blood due to compromised gut integrity[65,78]; this may explain why HIV viral loads are less consistently associated with immune activation in children than in adults.

In HIV-infected adults, *high* levels of CD8+ T-cell activation have been consistently predictive of mortality[62], while low activation is seen in HIV controllers[79]. We thus hypothesized that low CD8+ T-cell activation in normal ranges (<5%) would be predictive of good outcome. However, we found infants with <5% activated CD8+ T cells had a *higher* risk of mortality, suggesting very low activation in these infants is a marker of advanced HIV progression and immune exhaustion. Although few infants had very low activation, laboratory and clinical indicators were consistent with advanced HIV disease; these infants had higher median viral loads than the overall cohort, a greater proportion were WHO stage III/IV and they had very poor growth scores. Together, these data suggest very low levels of immune activation may indicate the loss of effective immune responses in end-stage infection. Consistent with this hypothesis, in a Ugandan study, HIV-specific T-helper responses were lower in children with lower T-cell activation, particularly CD8+ T-cell activation[75]. We did not have adequate specimens to evaluate virus-specific immune responses, and our phenotyping panel did not include markers of T-cell exhaustion or senescence, which would have been useful to further understand if the low activation in these children reflected T-cell exhaustion[80-82].

Few other pediatric studies have reported T-cell activation as a predictor of mortality. In a small US study, low absolute counts of CD8+CD38+ T-cells were predictive of mortality among children under two years old[64]. However, a South African study in children 4 months to 11 years old noted that high absolute count of CD8+CD38+ T-cells predicted mortality[73]. Importantly, these studies measured CD38+ alone as a marker of immune activation, which can

be problematic in pediatric studies because CD38+ expression on T-cells is normally high in healthy infancy and declines with age[83]. Other pediatric studies using HLA-DR expression as a marker of activation, without co-expression of CD38, have also reported contradictory results. An Italian study that included both treated and untreated children found that low CD8+HLA-DR+ percentages were predictive of immunologic and virologic worsening[84]. However, a US study found CD8+HLA-DR+ T-cell percentage of 5% or less among HIV-infected neonates, some of whom went on to start ART, was predictive of immunologic long-term non-progression[85]. In adults, CD8+ T-cell expression of HLA-DR in the absence of CD38 expression (CD8+CD38-HLA-DR+) has been found to be associated with long-term non-progression[86-90] and increased survival[62]; thus HLA-DR staining in the absence of CD38 may conflate two populations with different implications in HIV infection. Measurement of both CD38 and HLA-DR expression in our cohort avoided some of the issues of using either marker alone in a pediatric cohort.

The cohort, as a whole, had moderate to severe immunosuppression at baseline, and CD4% was associated with CD4+ but not CD8+ T-cell activation. In contrast, CD8+ T-cell activation prior to ART was associated with a slower rate of CD4% reconstitution in the first six months on ART, but not during the more distal time period between six months and two years. Similar results have been reported in adult ART cohorts[91,92] but were not seen in a Ugandan pediatric study.[67] While decline of T-cell activation is seen in most children after ART initiation[93,94], children with higher levels at baseline may have taken longer to normalize level of activation; the increased apoptosis and CD4 depletion accompanying high residual activation[95] may explain poor initial CD4 reconstitution. Rate of decrease of immune activation post-ART has been shown to be inversely related to the rate of CD4% and CD4 count reconstitution[93].

We did not find an association between pre-ART T-cell activation and viral load suppression. Few data exist on pre-ART T-cell activation as a predictor of virologic ART response in pediatric cohorts, and results are contradictory[72,84]; in contrast, residual activation of

CD8+[17,70,71] and CD4+ T-cells[96] after starting ART has been shown to be associated with viral load.

Strengths of our study include the narrow age range of infants at ART initiation, limiting age-related differences in CD38 expression and lymphocyte count, longitudinal 2 year follow-up and the availability of apoptosis and cell cycling markers to verify internal consistency of the data. Limitations include the previously noted lack of immune exhaustion and senescence data as well as the lack of T-cell function data. High mortality in the pre-ART period may have limited our ability to detect an association between pre-ART immune activation levels and post-ART treatment responses. As infants were started on ART as quickly as possible after HIV diagnosis, in accordance with Kenyan guidelines, the measurement of T-cell activation was limited to a single time-point prior to ART. This did not allow us to explore whether the activation we observed was representative of earlier levels or, particularly in the infants with <5% CD8+ T-cell activation, may have been preceded by higher levels.

Although T cell activation is consistently associated with an increased risk of mortality and poor treatment responses in adults, the relationship between pre-ART immune activation and infant outcomes is less clear-cut. Very low percentages of activated CD8+ T cells may be a marker for advanced HIV disease, rather than a predictor of contained immune response, while high levels of activation predict poor CD4 recovery. Strategies to augment immune responses and complement ART may be useful to improve outcomes in these vulnerable infants.

Table 2.1 Cohort characteristics at study enrollment.

	Data available	Median (IQR) or n (%)
Demographics and growth		
Infant age (months)	75	4.0 (3.3, 4.6)
Female	75	34 (45%)
Breastfed at enrollment	52	45 (87%)
Hospitalized at enrollment	75	50 (67%)
Weight-for-age Z-score	74	-2.7 (-3.8, -0.93)
Length-for-age Z-score	74	-2.0 (-3.3, -0.85)
HIV-related characteristics		
WHO stage III/IV	75	39 (52%)
CD4%	72	19 (15, 24)
HIV $\log_{10}$ viral load	74	6.6 (6.1, 7.2)
Mother received PMTCT	71	29 (41%)
Mother on ART for own health	71	7 (9.9%)
Follow-up of infant		
Infant initiated ART during the study	75	59 (79%)
Infant died before ART initiation	75	12 (16%)
Infant lost before ART initiation	75	4 (5%)

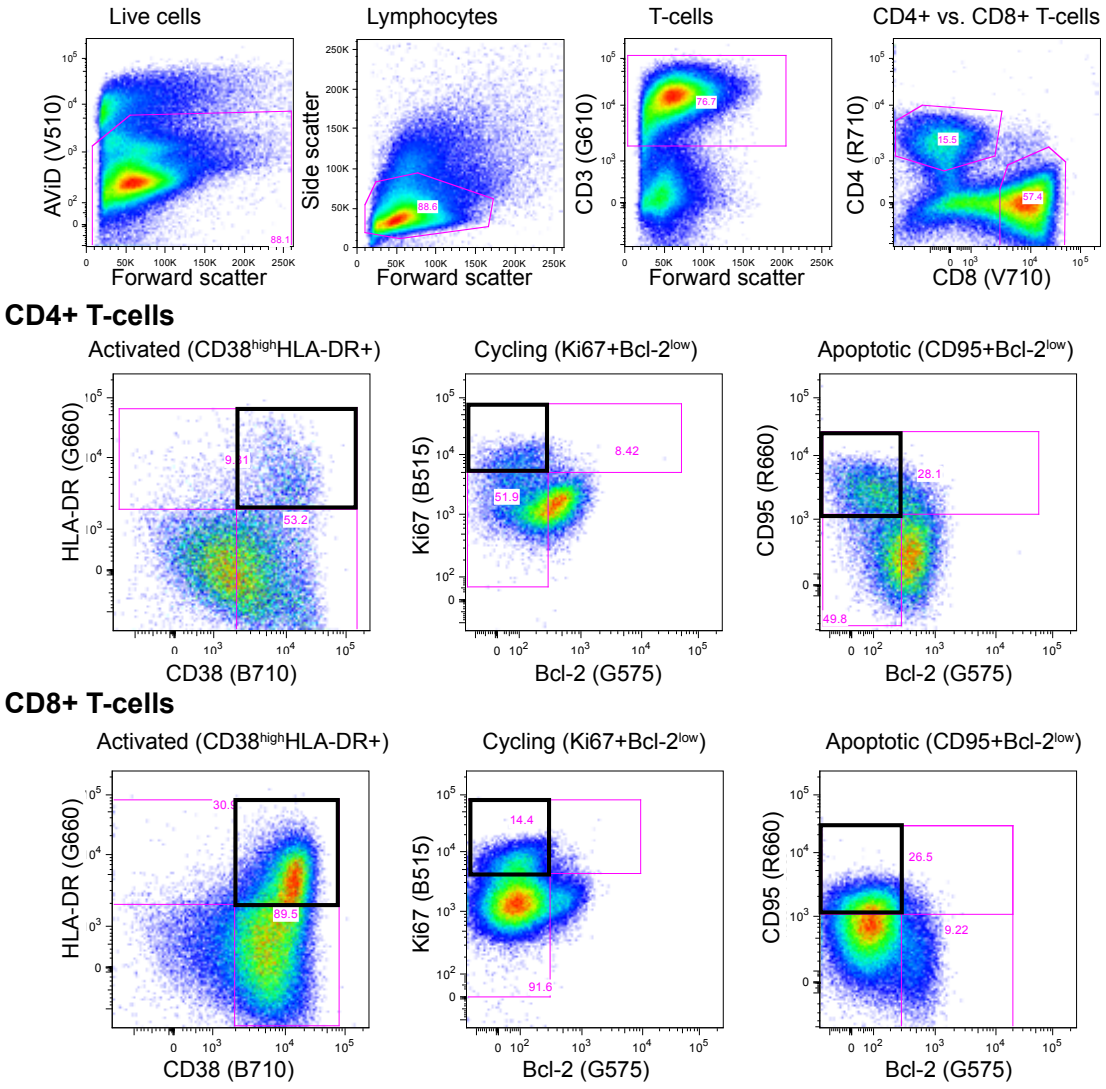


Figure 2.1 Flow cytometry T-cell phenotyping gating tree. Cells were collected on an LSR II instrument (BD Biosciences). Live cells were AViD negative; lymphocytes were selected using forward and side scatter within the live gate; T-cells were selected within the lymphocyte gate by CD3 expression. CD3+ T-cells were separated into CD4+ T-cells (CD4+CD8-) and CD8+ T-cells (CD4-CD8+). Activated, cycling and apoptotic subsets were quantified separately within the CD4+ and CD8+ T-cell populations.

Table 2.2 T-cell populations and phenotypic marker combinations.

T-cell population	CD4+ T-cells Median (IQR)	CD8+ T-cells Median % (IQR)
Activated ($CD38^{high}$/HLA-DR+)	3.3 (1.6, 5.5)	16.5 (9.9, 28.4)
Cycling (Ki67+/$Bcl2^{low}$)	4.5 (2.2, 9.8)	12.8 (7.3, 23.9)
Apoptosis-vulnerable (CD95+/$Bcl2^{low}$)	15.8 (9.2, 28.9)	13.1 (6.8, 21.6)

Table 2.3 Correlates of baseline T-cell activation among 75 ART-naive infants, assessed using Spearman's rank correlation.

	CD8+ T-cell activation ρ (p-value)	CD4+ T-cell activation ρ (p-value)
Age (days)	-0.211 (0.07)	0.007 (0.96)
Log_{10} HIV viral load	0.044 (0.71)	0.096 (0.42)
CD4%	0.152 (0.20)	-0.263 (0.03)
Weight-for-age Z-score	0.205 (0.08)	0.069 (0.56)
Length-for-age Z-score	0.198 (0.09)	0.177 (0.13)

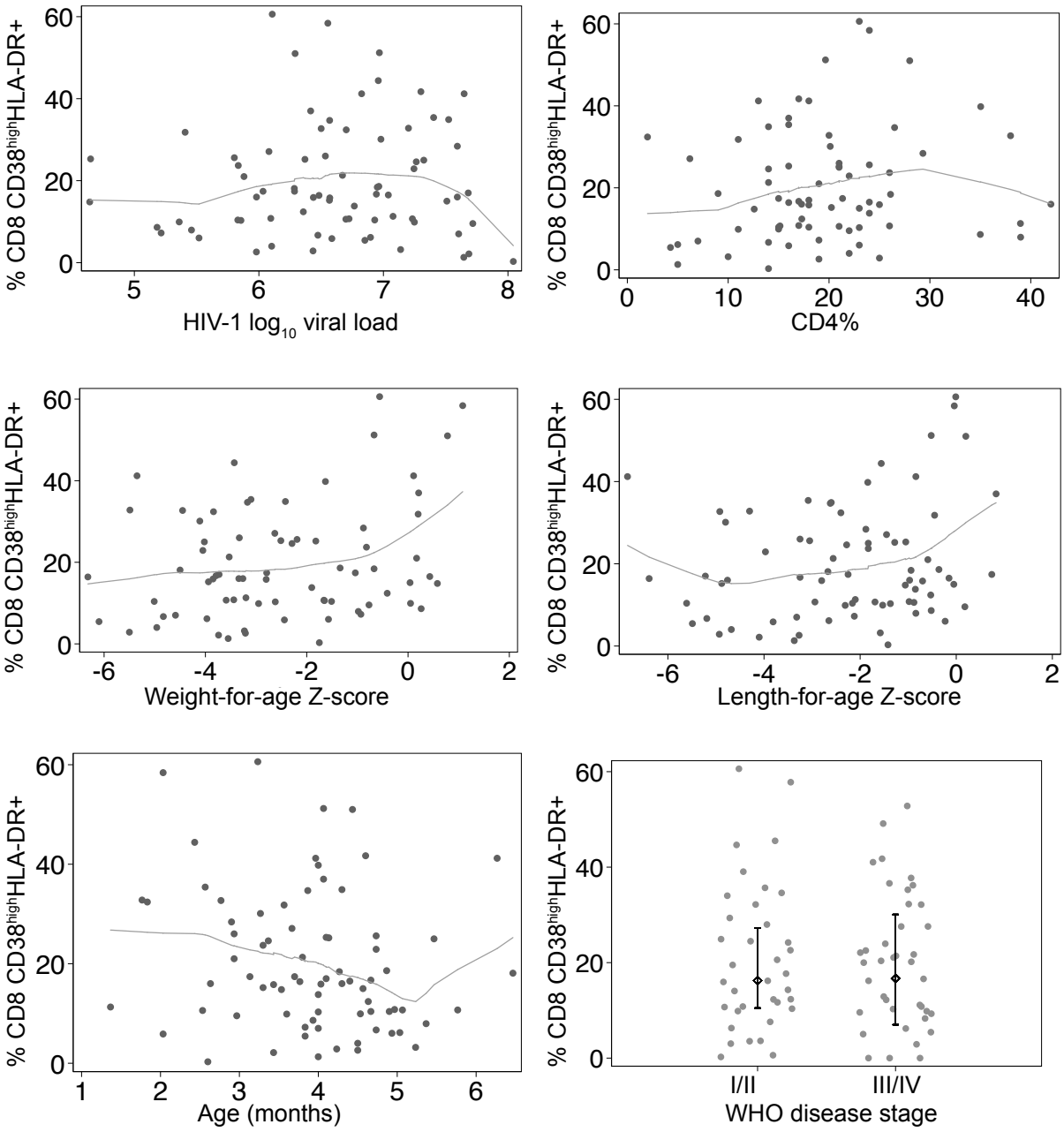


Figure 2.2 Scatterplots showing CD8+ T-cell activation (% CD8CD38^{high}/HLA-DR+) by baseline characteristics among 75 HIV-infected infants prior to ART initiation. Lines show lowess curves.

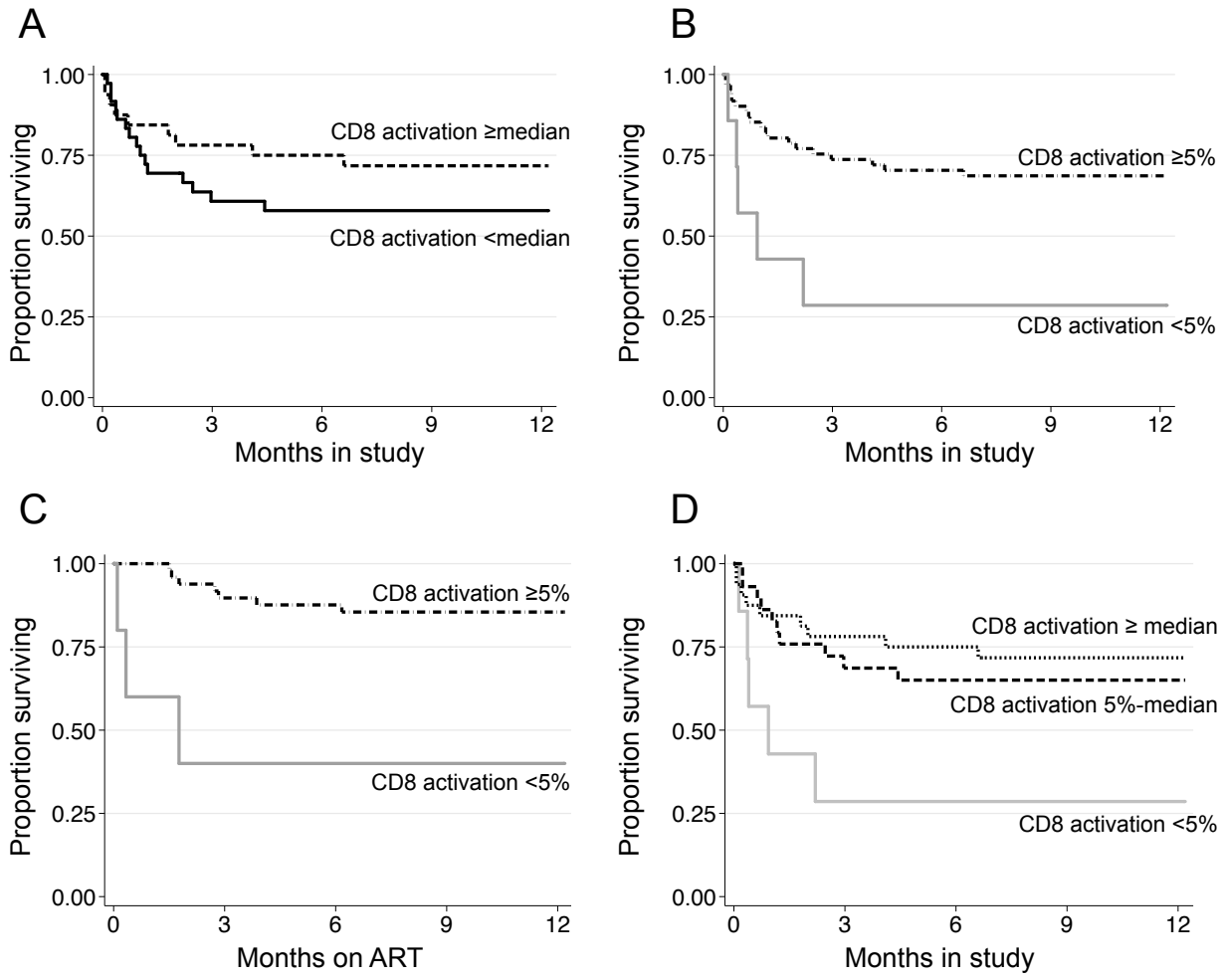


Figure 2.3 Kaplan-Meier curves showing 12-month mortality by CD8+ T-cell activation (%CD8+CD38^{high}HLA-DR+) at baseline. Panel A shows overall mortality by CD8 activation dichotomized at median (16.5%). Panel B shows overall mortality by activation dichotomized at 5%. Panel C shows mortality post-ART among the subset of infants who initiated ART, by activation dichotomized at 5%. Panel D shows overall mortality among infants in three categories of CD8+ T-cell activation: <5%; 5%-median; and ≥ median.

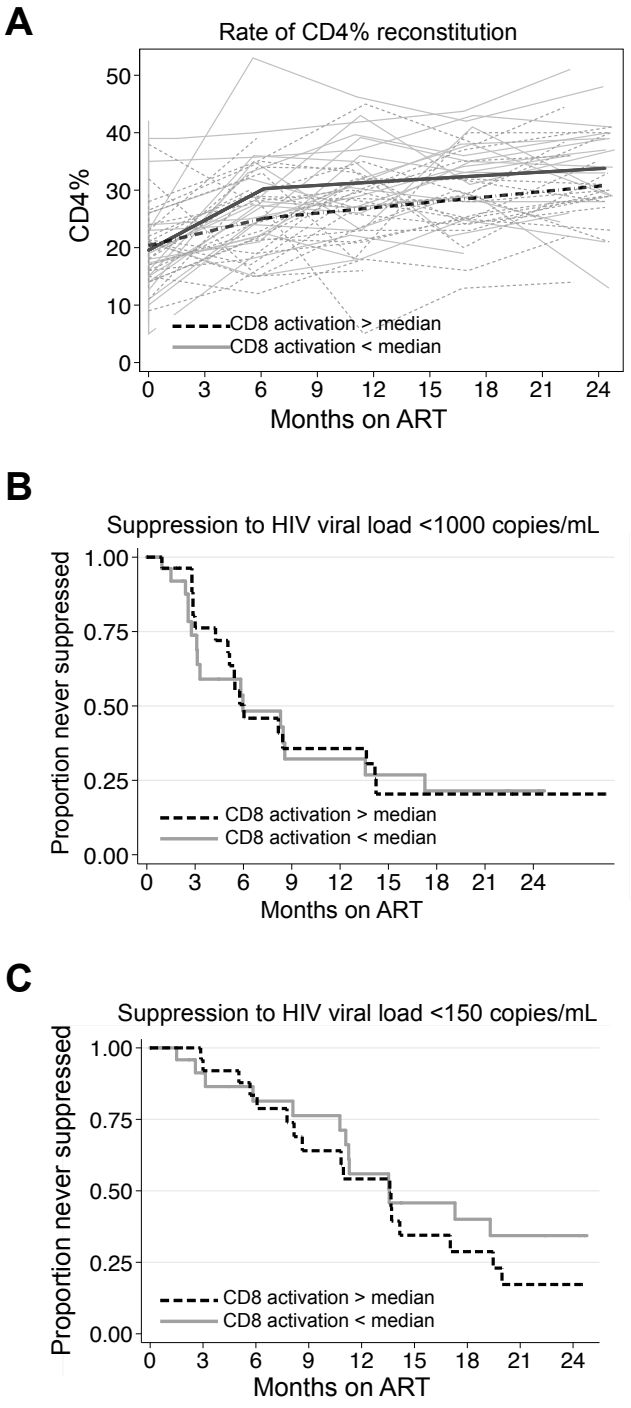


Figure 2.4 ART response by CD8+ T-cell activation at baseline. Panel A: Rate of CD4% reconstitution over two years of ART. Grey lines show individual trajectories, black lines show mean trajectories in each population. Solid lines represent infants with CD8 activation < median, dashed lines represent infants with CD8 activation > median. Panel B: Kaplan-Meier curve showing time to partial HIV viral load suppression to <1000 copies/mL. Panel C: Kaplan-Meier curve showing time to full HIV viral load suppression to <150 copies/mL (assay limit of detection).

Table 2.4 Supplementary T-cell phenotyping panel and antibody information. Unless otherwise noted, all antibodies were surface antibodies added prior to the permeabilization step.

Antibody	Vendor	Clone	Catalog number	Channel
CCR5-BV421	BD Biosciences	2D7/CCR5	562576	V450
CD45RA-BV605	BioLegend	HI100	304133	V610
CD27-BV655	BioLegend	O323	302827	V655
CD8-BV711	BD Biosciences	RPA-T8	563677	V710
Ki-67-FITCa	BD Biosciences	35/Ki-67	612472	B515
CD38-PerCP- Cy5.5	BD Biosciences	HIT2	551400	B710
Bcl-2-PE[a]	BD Biosciences	Bcl-2/100	340576	G575
CD3-PE-TR	Beckman Coulter	UCHT1	IM2705U	G610
HLA-DR-PE/Cy5	BioLegend	L243	307608	G660
CCR7-PE/Cy7	BioLegend	G043H7	353225	G780
CD95-APC	BD Biosciences	DX2	558814	R660
CD4-Ax700	BD Biosciences	RPA-T4	557922	R710

[a] Intracellular antibodies

Table 2.5 Supplementary Baseline characteristics and outcomes of seven infants with low CD8+ T-cell activation at enrollment (<5% CD8+CD38^{high}HLA-DR+).

% CD8 activation	Age (months)	WHO stage	HAZ	WAZ	log_{10} HIV viral load	CD4%	Infant's final status
3.2	4.8	II	-1.6	-3.2	7.1	10	Followed 2 years
1.3	3.7	III	-3.6	-3.5	7.6	5	Died post-HAART
2.9	3.8	III	-5.5	-5.5	6.9	25	Died post-HAART
2.1	3.4	III	-3.7	-3.7	7.7	missing	Died pre-HAART
4.0	4.5	III	-5.0	-5.0	6.1	22	Died pre-HAART
0.3	2.6	I	-1.4	-1.8	8.0	14	Died post-HAART
2.6	4.3	III	-3.2	-3.2	6.0	19	Followed 2 years

Chapter 3. Patterns of CD4+ T-cell reconstitution in infants initiating ART

3.1 BACKGROUND

HIV-infection disrupts T-cell subsets in a number of ways. The ratio of CD4+ to CD8+ T-cells is inverted[97] reflecting increased CD4+ destruction concurrent with increased proliferation of both CD4+ and CD8+ subsets. High T-cell activation[57] is accompanied by apoptosis of vulnerable cells[98-100] and is closely related to CD4+ T-cell depletion[58,95] and CD8+ T-cell clonal exhaustion[61,101]. During HIV infection, populations of long-lived central memory T-cells are depleted and their lifespans shortened[102,103] and T-cells increasingly shift toward an effector phenotype[104]. Skewed maturation leads to a less effective immune response[103] and eventually, homeostatic failure of central memory CD4+ T-cells leads to severe immunodeficiency (AIDS)[105].

CD4+ T-cell reconstitution following ART is thought to occur through a combination of thymic output, increased lifespan of existing cells, and proliferation of the CD4+ T-cells in circulation.[106] Adults, who have previously encountered a variety of antigens prior to immunodeficiency, primarily expand their memory T-cell subsets followed by a more modest increase in naive T-cells[107-109]. In contrast, in children an active thymus can contribute immune reconstitution through generation of new naive (CD45RA+) T-cells.[110-112] The extent of CD4+ T-cell recovery on ART is dependent in part on the extent of prior damage inflicted during HIV infection, including by high activation[91], inflammation[113] and fibrosis of the lymph nodes[114]. Central memory cells are preserved when ART is initiated during acute infection[115] but may not be fully restored by ART in chronic infection[116].

Discordant response to ART with immune recovery but no viral suppression has been observed in several pediatric cohorts[16,52,17,53]. Infants may be particularly likely to fall into this category because of their high thymic output. There are relatively few data on T-cell subset reconstitution among infants following ART.

3.2 METHODS

3.2.1 *Subjects*

HIV-infected Kenyan infants from the Optimizing Pediatric HAART study (described in Chapter 2.2.1) who started ART were included in this analysis. We determined memory CD4+ and CD8+ T-cell subsets among the 59 infants prior to ART initiation. A subset of 15 infants who completed at least 2 years of ART were selected on the basis of their response to ART and changes in their T-cell subsets described through two years of followup: 5 who had good CD4 and viral load response to ART (*responders),* 4 who had poor CD4 and viral load response to ART *(nonresponders)* and 6 with good CD4 response but poor viral load response *(discordant responders).*

3.2.2 *Sample selection*

Peripheral blood mononuclear cells (PBMCs) collected at baseline before ART initiation were analyzed for all infants. For the longitudinal subset, six-monthly PBMC samples were analyzed; where unavailable, the sample closest to the missing sample by date was selected.

3.2.3 *Laboratory methods*

T-cell phenotypes were analyzed by flow cytometry, according to a published protocol[74]. Cryopreserved PBMCs from infant blood samples were thawed into R10 buffer (RPMI 1640 with 25mM HEPES buffer and L-glutamine plus 10% FBS, 1% L-glutamine and 1% Penicillin-Streptomycin; Gibco BRL Life Technologies). PBMCs were stained with AViD dead cell stain (Life Technologies), then with a cocktail of T-cell phenotyping surface antibodies (Supplementary Table 2.1). All antibodies were titrated to appropriate concentrations before being used in the assay. Cells were permeabilized with FACS Lyse / FACS Perm II (BD Biosciences) and finally stained with intracellular antibodies. Stained cells were resuspended in

1% Paraformaldehyde (Electron Microscopy Sciences) solution for collection on an LSR II instrument (BD Biosciences).

3.2.4 *Phenotyping*

Samples collected on the LSR II were analyzed using FlowJo 9.7.6 (Tree Star, Ashland, Oregon). The T-cells (CD3+) were selected from the lymphocyte population and separated into CD4+ T-cells (CD4+CD8-) and CD8+ T-cells (CD4-CD8+). All subsets were quantified separately within the CD4+ and CD8+ T-cell population and expressed as percentages of the CD3+CD4+ or CD3+CD8+ population; absolute cell counts (number of cells per mm^3 blood) in each CD4+ subset were obtained by multiplying the cell subset proportion by the absolute CD4 T count at the same visit. Memory subsets consisted of naive (CD45RA+CCR7+CD27+), central memory (CD45RA-CCR7+CD27+), transitional memory (CD45RA-CCR7-CD27+), effector memory (CD45RA-CCR7-CD27-) and terminal effector (CD45RA+CCR7-CD27-) subsets (Figures 3.1 and 3.2). Activated ($CD38^{high}$/HLA-DR+) subsets were also quantified.

3.2.5 *Statistical methods*

Statistical analyses were performed using Stata 14.0 (StataCorp, College Station, Texas). Baseline characteristics of the full group of infants who initiated HAART and the subset who were longitudinally profiled were compared using the Mann-Whitney U-test. Associations between T-cell phenotype at baseline and response category were assessed using Mann-Whitney U-tests.

Changes in T-cell activation over two years of ART among children in each response category were modeled using linear mixed effects models with random intercepts. Slopes were assessed in two phases; one from ART initiation to six months and the other from six to 24 months. Change in T-cell activation in the cohort at large was modeled by weighting observations by each response category's sampling probability.

Correlates of residual T-cell activation on ART were assessed using Mann-Whitney U-tests and Spearman's rank correlation; pre-ART measurements of T-cell activation were excluded from this analysis.

Changes in the proportion of T-cells in each memory subset over two years of ART in each responder group were estimated using linear mixed effects models with random intercepts. Slopes were assessed in two phases; one from ART initiation to six months and the other from six to 24 months, mirroring the pattern of CD4% reconstitution in the cohort. Percentages in each memory cell subset were transformed using log or square root transformation, as appropriate, to improve normality of the data, and model estimates were back-transformed for presentation in figures.

In order to determine whether CD4 reconstitution was driven by the same memory subsets among responders and discordant responders, changes in raw CD4+ T-cell counts (cells/mm^3) in each memory subset over two years of ART were modeled using a linear mixed effects model. Interaction terms were included to assess differences in rate of CD4+ T-cell repopulation in each phase by responder type. CD4+ T-cell counts in each memory cell subset were transformed using log or square root transformation, as appropriate, to improve normality of the data, and model estimates were back-transformed for presentation in figures.

3.3 RESULTS

3.3.1 *Cohort*

Of the 59 infants who initiated ART, 12 died post-ART, 8 were withdrawn or lost to followup at <2 years, and 39 completed two full years of followup. Among infants with 2 years of follow-up, responses to ART fell into three patterns: ***responders***, those who fully suppressed HIV viral load to undetectable levels and reconstituted CD4 to 25% within one year of ART (n=22), ***nonresponders*** who failed to suppress HIV viral load or reconstitute CD4 to 25% within

one year (n=4), and ***discordant responders*** who reconstituted CD4 but failed to suppress viral load by one year (n=13). T-cell activation and memory subset distribution at baseline did not predict response category (data not shown). The longitudinal subset consisted of all four nonresponders, six discordant responders and five responders with complete followup.

Among the overall set of 59 infants who initiated ART, median age at ART initiation was 4.0 months and median HIV viral load pre-ART was 6.7 $\log_{10}$ copies/mL. At baseline prior to ART initiation, 45% of infants were WHO stage III/IV. Growth status in the cohort was poor prior to ART initiation, with median length-for-age (LAZ) and weight-for-age Z-scores (WAZ) -2.1 and -2.4, respectively. The subset of infants included in the longitudinal subset had lower viral loads (median 6.3 $\log_{10}$ copies/mL) and better growth (median LAZ=-1.1 and median WAZ=-1.6) at baseline than the cohort at large; differences were not statistically significant.

3.3.2 *Memory T-cell subsets at baseline*

Prior to ART initiation, in the CD4+ T cell subset, the majority of cells were naïve (median=63%, IQR 30-75%, Table 3.2), and a large proportion were central memory (17.1%, IQR 11.9-25.3%). Transitional memory, effector memory, and terminal effector cell subsets comprised <10% of CD4+ T-cells in most infants.

In contrast, only a small subset in the CD8+ T cell subset were naive (8.5%, IQR 5.4-15.4%) or central memory (3.0%, IQR 1.6-6.7%), and the majority of cells were of the more differentiated memory subsets: transitional memory (median 25%, IQR 11.3-37.6%), effector memory (17.7%, IQR 12.5-31.3%) and terminal effector (12.3%, IQR 6.2-28.3%) phenotypes.

3.3.3 *Changes in T-cell activation on ART*

CD4+ T-cell activation did not change significantly in either the first 6 months or between 6-24 months in any of the responder groups (Table 3.3, Figure 3.3A). No association was

observed between CD4+ T-cell activation and detectable viral load (p=0.45) or CD4% (ρ=0.18, p=0.23) at timepoints post-ART initiation.

The weighted model estimated a 0.83-fold change in CD8+ T-cell activation per month on ART in the first six months (0.83-fold change per month on ART (95% CI 0.75-0.92), p<0.001, Figure 3.3B); and a 1.03-fold change per month between 6-24 months (95% CI 1.00-1.06), p=0.05). Residual CD8+ T-cell activation was associated with detectable viral load post-ART (p=0.005) and there was a weak but significant correlation between higher CD8+ T-cell activation and lower CD4% (ρ=-0.36, p=0.03). CD8+ T-cell activation decreased significantly during the first six months on ART in all responder groups, followed by an increase between 6-24 months, though this was not significant within each group (Table 3.3).

3.3.4 *Changes in T-cell memory subsets on ART*

CD4+ T-cell count rose rapidly in the responder and discordant groups following ART initiation (data not shown); no significant change in CD8+ T-cell count was observed.

The weighted model estimated a 1.14-fold increase in naive CD8+ T-cells per month in the first six months on ART (95% CI 1.02-1.26, p=0.03) followed by a 0.96-fold decrease per month from 6-24 months on ART (95% CI 0.93-0.99, p=0.008). An increase in the proportion of CD8+ T-cells in the naive subset was observed in discordant responders in the first six months on ART (1.12-fold change per month (95% CI 1.04-1.20), p=0.003, Table 3.3); point estimates in the two other response categories were also positive but the slopes were not significant. Between 6-24 months, however, the naive CD8+ T-cell proportion decreased significantly among discordant responders (0.94-fold change per month (95% CI 0.91-0.97), p<0.001). No significant changes in other CD8+ T-cell memory subsets were observed in any responder category (data not shown). There were no significant changes observed in CD4+ memory T-cell proportions in either the first six months or from six-24 months on ART in any responder category (data not shown).

Because CD4+ T-cell count increased dramatically following ART among responder and discordant responder infants, absolute CD4+ subset counts were modeled in addition to proportions. From these models, good responders had a significant increase in naive CD4+ T-cell count over the first six months on ART (1.16-fold change per month for an average infant (95%1.05-1.26), p=0.003; Table 3.4, Figure 3.4), while discordant responders had no increase in naive CD4+ T-cells (p=0.48). The difference in slopes was significant (p=0.02). There was no significant change in naive CD4+ T-cell count in either group from 6-24 months.

The absolute number of CD4+ central memory T-cells increased among discordant responders in the first six months on ART (1.14-fold change per month for an average infant (95% CI 1.03-1.24), p=0.009, Figure 3.4). Good responders showed a non-significant increase in the same time period (1.08-fold change per month for an average infant (95% CI 0.96-1.20), p=0.2) and the difference in slopes was not significant (p=0.49). There was no significant change in central memory CD4+ T-cell count between 6 and 24 months. No significant changes in other CD4+ T-cell memory subset counts were observed in either the first six months on ART or between 6-24 months.

3.4 DISCUSSION

In this cohort of early-treated HIV-infected infants, there were varied patterns of viral and immune response to ART. Half the infants completing two years of ART suppressed HIV viral load to undetectable levels and reconstituted CD4 to 25% by 12 months, but a third reconstituted CD4% but failed to suppress virus. The proportion of CD8+ T-cells activated decreased substantially in the first six months on ART. The proportion of CD8+ naïve T-cells increased substantially in first 6 months post-ART with smaller declines in the subsequent 18 months. In the CD4 compartment, no significant changes in proportions were found, but the absolute number of central memory CD4+ T-cells increased significantly among responders and

discordant responders in the first six months on ART, and the absolute number of naïve cells increased among responders.

Similar to findings in other pediatric HIV cohorts, at baseline prior to ART initiation, we found a smaller proportion of both CD4+ and CD8+ T-cells were naive compared to HIV-uninfected African infants[68,94,117]. Central memory cells constituted the largest CD4+ memory subset in our study. This contrasts with data from a large Zambian cohort[94] in which CD4+ central memory cells were the smallest subset. The proportion of CD4+ terminal effector T-cells was also substantially smaller in our cohort, indicating an overall less differentiated phenotype. This difference may be partly explained by the inclusion of CD27 as a confirmatory marker in our phenotyping assay, resulting in a smaller proportion of T-cells designated terminal effector than had we used CD45RA and CCR7 alone; and by the age of the cohorts. In the Zambian study, only 10 children were ≤12 months old, and of these the median age was 9.6 months, compared to a median age at baseline of 4.0 months in our study. This is a substantial difference given rapid disease progression among HIV-infected African infants.

In longitudinally followed infants in the study, CD8+ T-cell activation decreased rapidly during the first six months of ART, but then increased between six and 24 months; persistent CD8+ T-cell activation was associated with detectable viral load. The decline of T-cell activation is consistent with studies in other adult and pediatric cohorts[93,94], as is the association between CD8+ T-cell activation and lack of viral suppression among treated children[17,70,71]. The increase in CD8+ T-cell activation between six and 24 months on ART we observed may be due to the changing pathogen exposures in infants as they become more mobile and cease breastfeeding; increased gut permeability[118] and microbial translocation across the gut mucosa[78,118] in the first year of life have been described in African infants regardless of HIV status and may drive immune activation in this period.

We observed a significant increase in the proportion of naive CD8+ T-cells over the first six months on ART, followed by a decrease between six and 24 months. A similar pattern was observed in the number of naive CD4+ T-cells among good responders. Changes in the size of the naive subset accompany both ART initiation[111] and normal maturation of the immune system[24,117]. In healthy children, the proportion of naïve cells declines rapidly in early life[24,117], from >90% of T-cells naive in neonates to 75% of CD8+ T-cells and 67% of CD4+ T-cells between 12 and 24 months of age in an African reference population[117]. Regardless of HIV infection, proportions of T-cells naive at a given age tend to be lower among children in resource-limited than high-resource settings,[68,94,117] likely reflecting higher pathogen exposure. With ART, proportions of naïve CD8+ T-cells increased substantially in the first 6 months, however not to levels expected in healthy children of similar ages suggesting accelerated decline of naïve population due to early HIV infection. Earlier ART prior to symptomatic disease may preserve more naïve cells and be useful to enable immune responses to vaccines or new infections.

We observed some differences in the patterns of CD4+ T-cell reconstitution by responder type. While good responders had increased numbers of naive CD4+ T-cells in the first six months, discordant responders had a slight but not significant decrease in the same time period, possibly due to their persistent viral loads. Both good and discordant responders showed increased numbers of central memory CD4+ T-cells, as expected during ART[94,119,120]. The increase in naive and central memory CD4+ T-cell count suggests that immune repopulation in the responder and discordant responder infants resulted in a shift toward a less differentiated T-cell phenotype, although no significant changes in either the proportion or count of effector memory and terminal effector T-cells were seen.

Our study was exploratory and limited by a small sample size, particularly in the nonresponder group. We did not adjust for multiple comparisons. Our study lacked an age-

matched control group of infants to distinguish between the effects of infant age and ART on changes in T-cell subsets. Strengths of the study included the narrow age range of the infants and the extended follow-up.

Infants in our study recovered naive and central memory T-cells in the first six months on ART and their CD8+ T-cell activation was reduced, consistent with changes seen in older children starting ART. Differences in the pattern of recovery were observed between virologic responders and non-responders, suggesting that infants who respond immunologically but not virologically to ART may continue to have skewed maturation of T-cells compared to virologic responders.

Table 3.1 Baseline characteristics of HIV-infected infants prior to ART initiation. Characteristics are shown for the whole cohort of 59 children initiating ART and in the subset whose T-cell subsets were profiled longitudinally over two years of ART.

	Infants initiating ART (n=59) Median (IQR)	**Longitudinal subset (n=15)** Median (IQR)	**p-value**
Age at ART initiation (days)	123 (99, 142)	133 (99, 152)	0.50
HIV viral load pre-ART	6.7 (6.0, 7.1)	6.3 (5.8, 6.7)	0.05
CD4% pre-ART	19 (15, 24)	21 (16, 26)	0.32
WHO stage III/IV pre-ART	36 (45%)	5 (33%)	0.08
Length-for-age Z-score	-2.1 (-3.3, -0.84)	-1.1 (-2.6, -0.53)	0.08
Weight-for-age Z-score	-2.4 (-3.7, -0.82)	-1.6 (-3.3, -0.41)	0.09
PI-based HAART	26 (44%)	4 (27%)	0.34
NVP-based HAART	33 (56%)	11 (73%)	

Table 3.2 Distribution of T-cell subset percentages among 59 infants prior to initiation of ART.

CD4+ T-cell subsets	**Median**	**(IQR)**
Naïve (CD45RA+CCR7+CD27+)	62.6	(30.4, 75.3)
Central memory (CD45RA-CCR7+CD27+)	17.1	(11.9, 25.3)
Transitional memory (CD45RA-CCR7-CD27+)	6.1	(3.3, 13.0)
Effector memory (CD45RA-CCR7-CD27-)	5.3	(2.0, 12.1)
Terminal effector (CD45RA+CCR7-CD27-)	0.5	(0.2, 1.3)
Activated ($CD38^{high}$/HLA-DR+)	3.4	(1.6, 5.3)
CD8+ T-cell subsets	**Median**	**(IQR)**
Naïve (CD45RA+CCR7+CD27+)	8.5	(5.4, 15.4)
Central memory (CD45RA-CCR7+CD27+)	3.0	(1.6, 6.7)
Transitional memory (CD45RA-CCR7-CD27+)	25.0	(11.3, 37.6)
Effector memory (CD45RA-CCR7-CD27-)	17.7	(12.5, 31.3)
Terminal effector (CD45RA+CCR7-CD27-)	12.3	(6.2, 28.3)
Activated ($CD38^{high}$/HLA-DR+)	16.7	(9.9, 26.0)

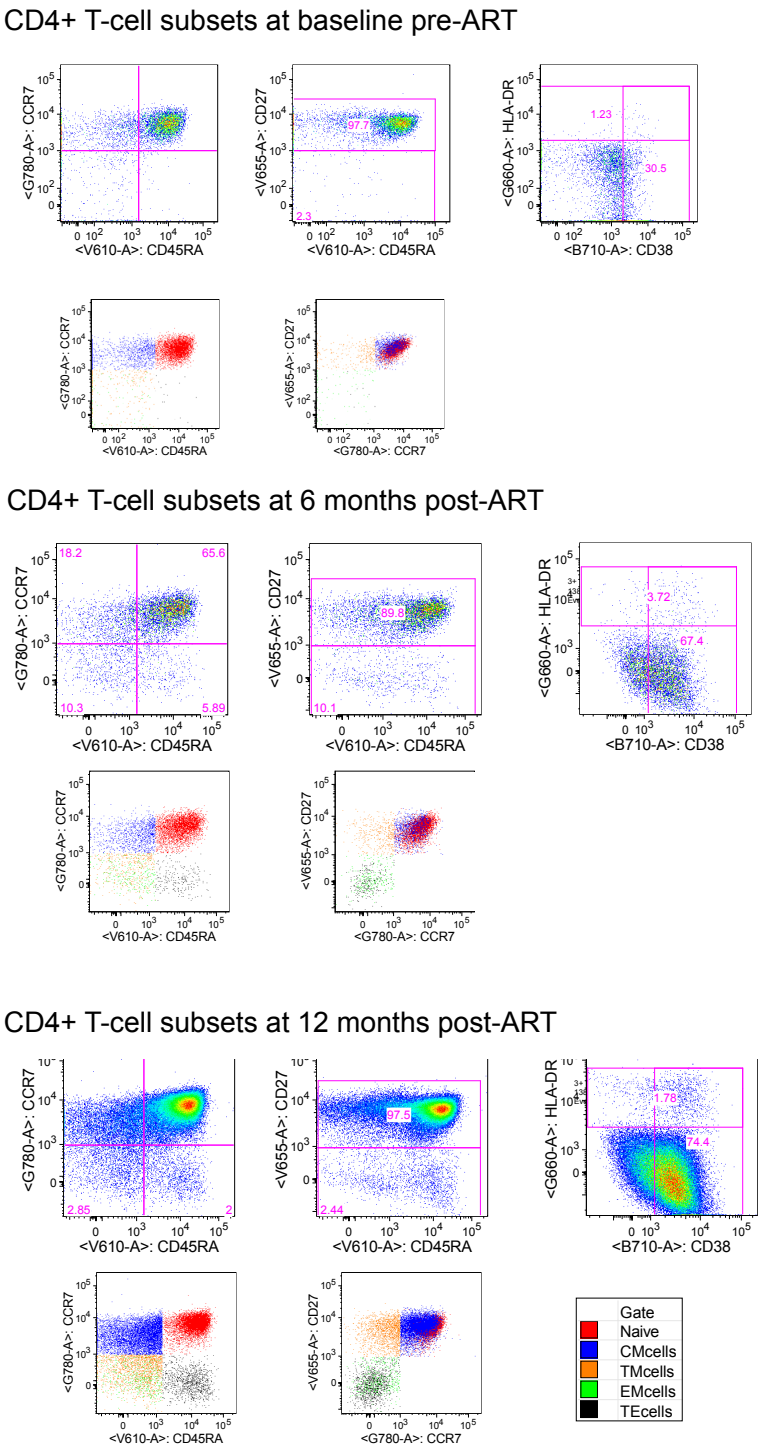


Figure 3.1 Flow cytometry plots showing changes in CD4+ T-cell populations over 12 months of ART in a single "good responder" subject. Upper rows at each time point show two-dimensional flow cytometry plots; lower plots show overlaid naive (shown in red; CD45RA+CCR7+CD27+), central memory (CM cells, blue; CD45RA-CCR7+CD27+) transitional memory (TM cells, orange; CD45RA-CCR7-CD27+), effector memory (green; EM cells, CD45RA-CCR7-CD27-) and terminal effector (TE cells, black; CD45RA+CCR7-CD27-) populations.

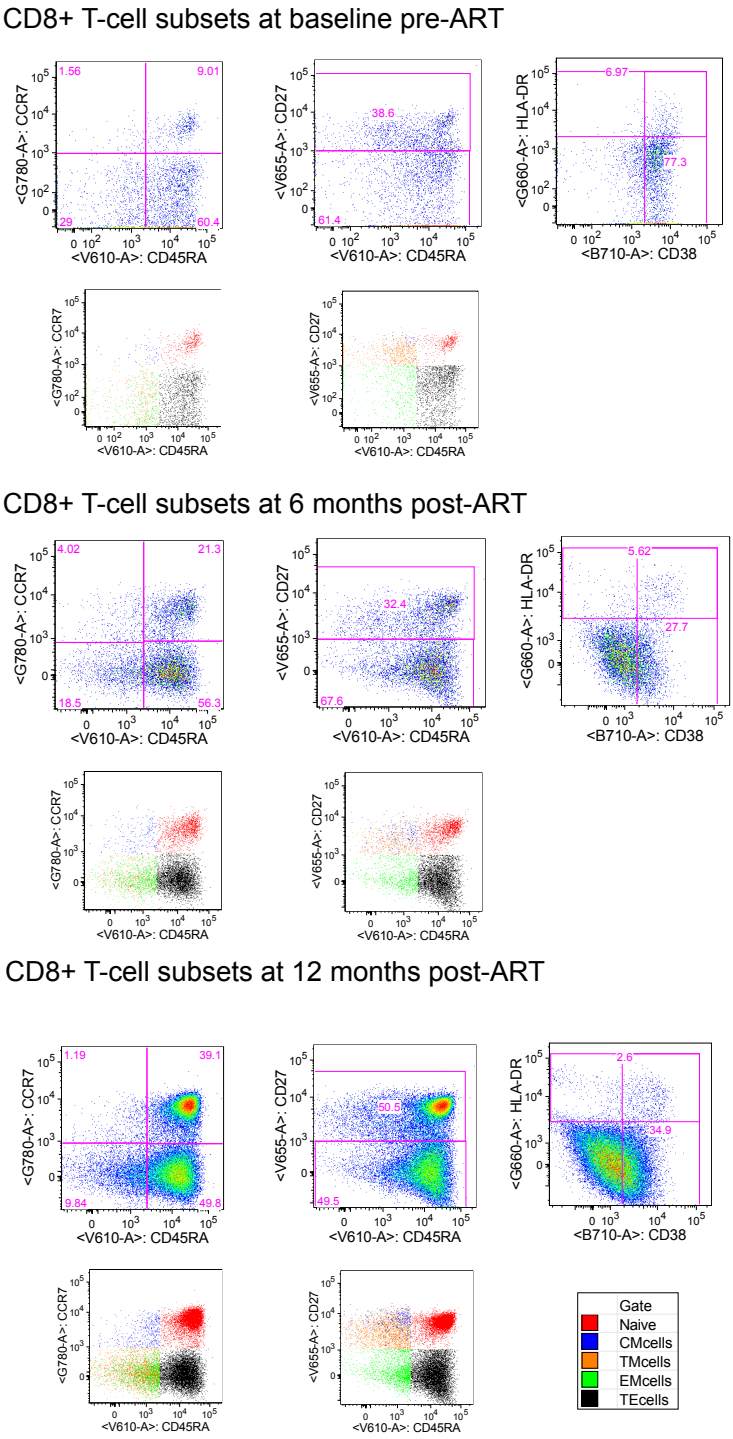


Figure 3.2 Flow cytometry plots showing changes in CD8+ T-cell populations over 12 months of ART in a single "good responder" subject. Upper rows at each time point show two-dimensional flow cytometry plots; lower plots show overlaid naive (shown in red; CD45RA+CCR7+CD27+), central memory (CM cells, blue; CD45RA-CCR7+CD27+) transitional memory (TM cells, orange; CD45RA-CCR7-CD27+), effector memory (EM cells, green; CD45RA-CCR7-CD27-) and terminal effector (TE cells, black; CD45RA+CCR7-CD27-) populations.

Table 3.3 Changes in T-cell proportions over two years of ART, modeled using linear mixed effects models. One slope was estimated from 0-6 months on ART and another from 6-24 months on ART. Proportions were log-transformed to improve normality of the data.

	Slope 0-6 months		Slope 6-24 months	
	β_1 (95% CI)	p-value	β_2 (95% CI)	p-value
CD4+ T-cell activation (log(%))				
Nonresponders	-0.02 (-0.19, 0.15)	**0.84**	0.04 (-0.04, 0.11)	**0.35**
Discordant responders	-0.06 (-0.18, 0.07)	**0.40**	0.04 (-0.01, 0.09)	**0.09**
Responders	0.06 (-0.06, 0.17)	**0.35**	0.00 (-0.04, 0.05)	**0.91**
Overall weighted estimate	*0.01 (-0.07, 0.09)*	***0.83***	*0.02 (-0.01, 0.05)*	***0.25***
CD8+ T-cell activation (log(%))				
Nonresponders	-0.18 (-0.36, 0.00)	**0.05**	0.07 (-0.01, 0.15)	**0.08**
Discordant responders	-0.10 (-0.21,0.01)	**0.08**	0.03 (-0.01, 0.07)	**0.14**
Responders	-0.25 (-0.38, -0.11)	**0.001**	0.03 (-0.02, 0.09)	**0.21**
Overall weighted estimate	*-0.18 (-0.25, 0.11)*	***<0.001***	*0.03 (0.00, 0.06)*	***0.06***
CD8+ naive (log(%))				
Nonresponders	0.05 (-0.08, 0.18)	**0.47**	0.01 (-0.05, 0.07)	**0.67**
Discordant responders	0.12 (0.04, 0.20)	**0.003**	-0.06 (-0.09, -0.03)	**<0.001**
Responders	0.13 (-0.06, 0.31)	**0.18**	-0.03 (-0.10, 0.04)	**0.47**
Overall weighted estimate	*0.14 (0.02, 0.26)*	***0.03***	*-0.04 (-0.07, -0.01)*	***0.008***

Table 3.4 Rate of change in CD4+ T-cell subset counts among infants who reconstituted CD4 to >25% by one year of ART. Changes in subset size were modeled using linear mixed effects models, with one slope estimated from 0-6 months on ART and another from 6-24 months on ART. P-values test the equality of slopes in each time period. Proportions were log-transformed to improve normality of the data.

	Discordant responders β (95% CI)	Good responders β (95% CI)	p-value
Naive CD4+ T-cell count (log(cells/mm3))			
Slope 0-6 months	-0.05 (-0.20, 0.09)	0.16 (0.05, 0.26)	**0.02**
Slope 6-24 months	-0.003 (-0.08, 0.03)	-0.04 (-0.08, 0.00)	**0.03**
Central memory CD4+ T-cell count (log(cells/mm^3))			
Slope 0-6 months	0.14 (0.03, 0.24)	0.08 (-0.04, 0.20)	**0.49**
Slope 6-24 months	-0.04 (-0.07, 0.00)	-0.02 (-0.06, 0.03)	**0.60**

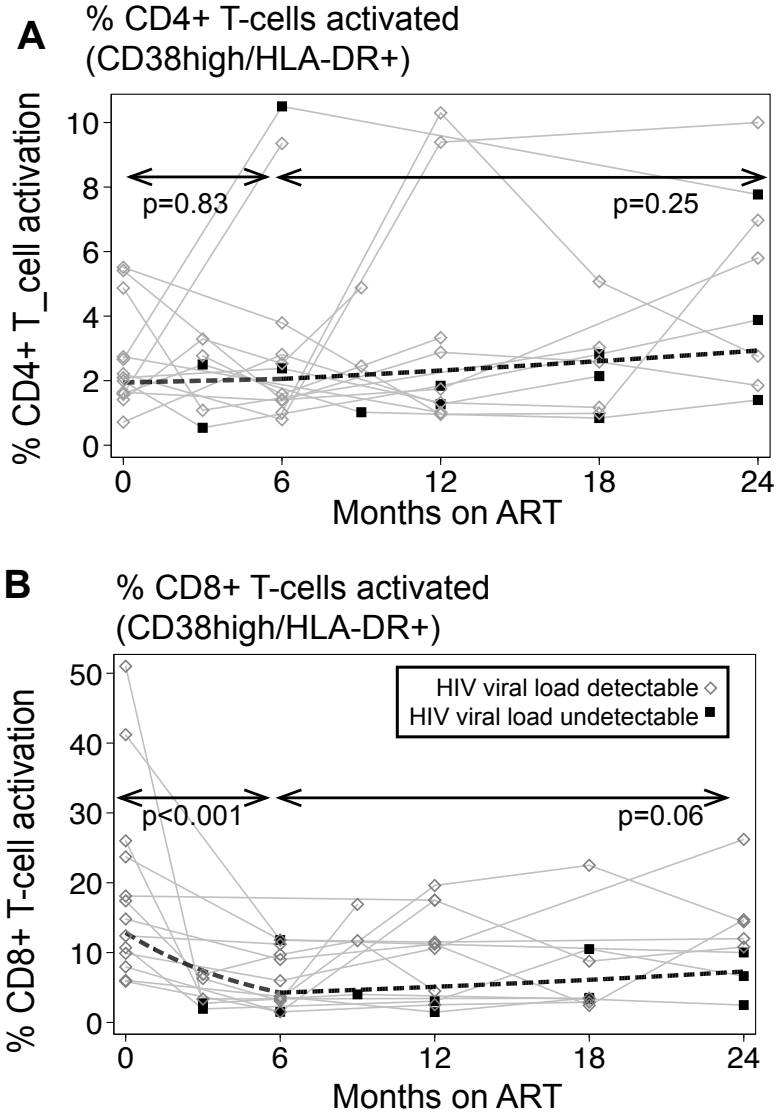


Figure 3.3 Changes in T-cell activation over two years of ART. Changes were modeled in two phases: 0-6 months and 6-24 months, using linear mixed effects models. Grey lines show individual trajectories among 15 infants who were profiled longitudinally; black lines show cohort mean trajectories, weighted by sampling probability. Proportions in each T-cell subset were log-transformed to improve normality, then trajectories were back-transformed for visual presentation. P-values refer to the change over time in each phase in the weighted model.

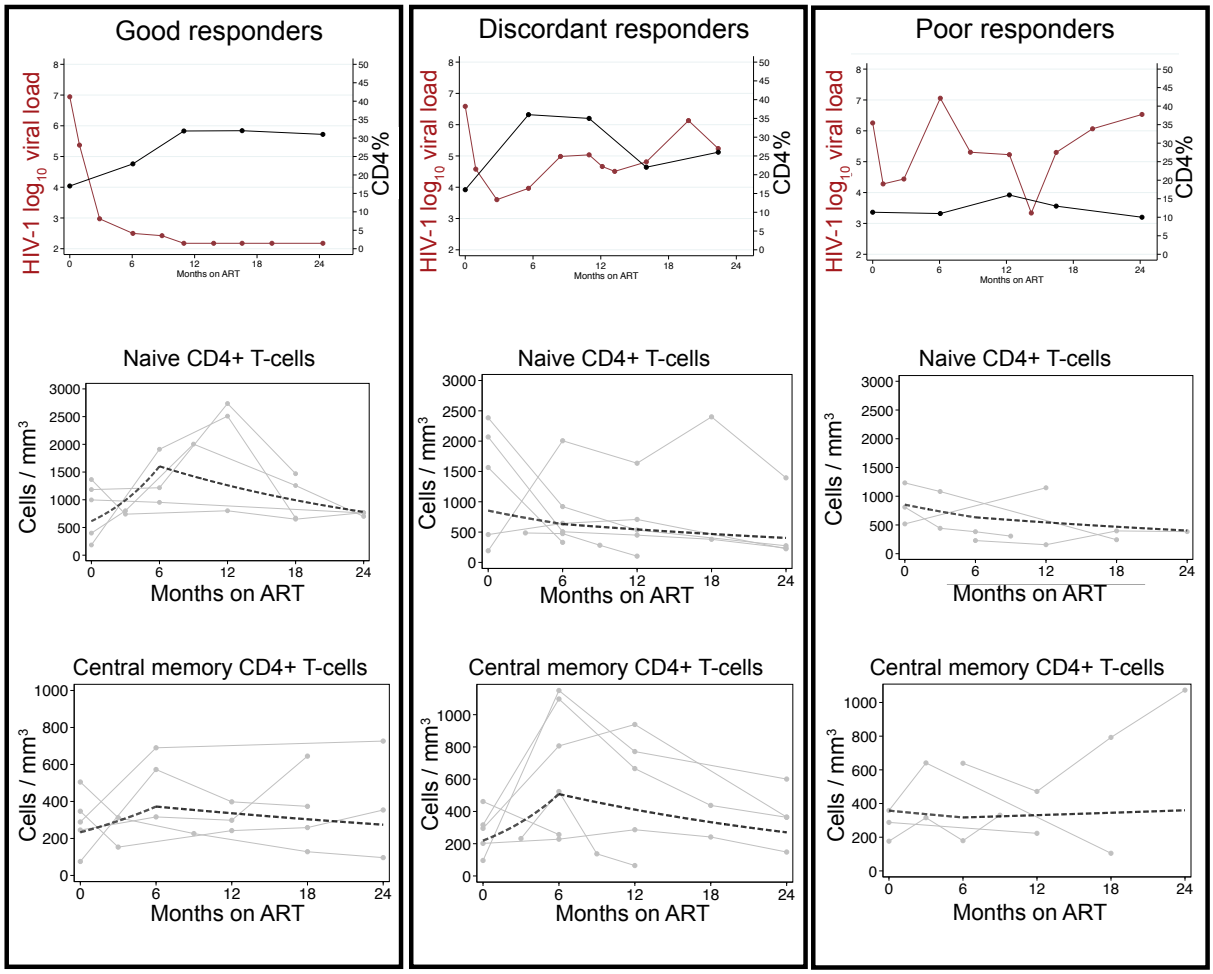


Figure 3.4 Trajectories of infant CD4+ T-cell subset counts over two years of ART by response profile. Good responders (n=5) fully suppressed HIV viral load to undetectable levels and reconstituted CD4 to 25% within one year of ART, poor responders (n=4) failed to suppress HIV viral load or reconstitute CD4 to 25% within one year, and discordant responders (n=6) reconstituted CD4 but failed to suppress viral load by one year (n=6).

BIBLIOGRAPHY

1. Obimbo EM, Wamalwa D, Richardson B, et al. Pediatric HIV-1 in Kenya: pattern and correlates of viral load and association with mortality. *Journal of Acquired Immune Deficiency Syndromes.* 2009;51(2):209-215.
2. Newell ML, Coovadia H, Cortina-Borja M, Rollins N, Gaillard P, Dabis F. Mortality of infected and uninfected infants born to HIV-infected mothers in Africa: a pooled analysis. *Lancet.* 2004;364(9441):1236-1243.
3. Shearer WT, Quinn TC, LaRussa P, et al. Viral load and disease progression in infants infected with human immunodeficiency virus type 1. Women and Infants Transmission Study Group. *New England Journal of Medicine.* 1997;336(19):1337-1342.
4. Richardson BA, Mbori-Ngacha D, Lavreys L, et al. Comparison of human immunodeficiency virus type 1 viral loads in Kenyan women, men, and infants during primary and early infection. *Journal of Virology.* 2003;77(12):7120-7123.
5. Palumbo PE, Raskino C, Fiscus S, et al. Predictive value of quantitative plasma HIV RNA and CD4+ lymphocyte count in HIV-infected infants and children. *JAMA.* 1998;279(10):756-761.
6. Natural history of vertically acquired human immunodeficiency virus-1 infection. The European Collaborative Study. *Pediatrics.* 1994;94(6 Pt 1):815-819.
7. Jain V, Byonanebye DM, Amanyire G, et al. Successful antiretroviral therapy delivery and retention in care among asymptomatic individuals with high CD4+ T-cell counts above 350 cells/mul in rural Uganda. *AIDS.* 2014;28(15):2241-2249.
8. El-Khatib Z, Ekstrom AM, Coovadia A, et al. Adherence and virologic suppression during the first 24 weeks on antiretroviral therapy among women in Johannesburg, South Africa - a prospective cohort study. *BMC Public Health.* 2011;11:88.
9. Reepalu A, Balcha TT, Skogmar S, et al. High rates of viral suppression in a cohort of HIV-positive adults receiving ART in Ethiopian health centers irrespective of concomitant tuberculosis. *Journal of the International AIDS Society.* 2014;17(4 Suppl 3):19612.
10. Mulu A, Liebert UG, Maier M. Virological efficacy and immunological recovery among Ethiopian HIV-1 infected adults and children. *BMC Infect Dis.* 2014;14:28.
11. Ruel TD, Kakuru A, Ikilezi G, et al. Virologic and immunologic outcomes of HIV-infected Ugandan children randomized to lopinavir/ritonavir or nonnucleoside reverse transcriptase inhibitor therapy. *Journal of Acquired Immune Deficiency Syndromes.* 2014;65(5):535-541.
12. Meyers TM, Yotebieng M, Kuhn L, Moultrie H. Antiretroviral therapy responses among children attending a large public clinic in Soweto, South Africa. *Pediatric Infectious Disease Journal.* 2011;30(11):974-979.
13. van Dijk JH, Sutcliffe CG, Munsanje B, et al. HIV-infected children in rural Zambia achieve good immunologic and virologic outcomes two years after initiating antiretroviral therapy. *PloS One.* 2011;6(4):e19006.
14. Aboulker JP, Babiker A, Chaix ML, et al. Highly active antiretroviral therapy started in infants under 3 months of age: 72-week follow-up for CD4 cell count, viral load and drug resistance outcome. *AIDS.* 2004;18(2):237-245.
15. Haberer J, Mellins C. Pediatric adherence to HIV antiretroviral therapy. *Current HIV/AIDS Reports.* 2009;6(4):194-200.
16. Ghaffari G, Passalacqua DJ, Caicedo JL, Goodenow MM, Sleasman JW. Two-year clinical and immune outcomes in human immunodeficiency virus-infected children who reconstitute CD4 T cells without control of viral replication after combination antiretroviral therapy. *Pediatrics.* 2004;114(5):e604-611.

17. Anselmi A, Vendrame D, Rampon O, Giaquinto C, Zanchetta M, De Rossi A. Immune reconstitution in human immunodeficiency virus type 1-infected children with different virological responses to anti-retroviral therapy. *Clinical and Experimental Immunology.* 2007;150(3):442-450.
18. Lewis J, Walker AS, Castro H, et al. Age and CD4 count at initiation of antiretroviral therapy in HIV-infected children: effects on long-term T-cell reconstitution. *Journal of Infectious Diseases.* 2012;205(4):548-556.
19. Newell ML, Patel D, Goetghebuer T, Thorne C, European Collaborative S. CD4 cell response to antiretroviral therapy in children with vertically acquired HIV infection: is it associated with age at initiation? *Journal of Infectious Diseases.* 2006;193(7):954-962.
20. Picat MQ, Lewis J, Musiime V, et al. Predicting patterns of long-term CD4 reconstitution in HIV-infected children starting antiretroviral therapy in sub-Saharan Africa: a cohort-based modelling study. *PLoS Medicine.* 2013;10(10):e1001542.
21. De Rossi A, Walker AS, Klein N, De Forni D, King D, Gibb DM. Increased thymic output after initiation of antiretroviral therapy in human immunodeficiency virus type 1-infected children in the Paediatric European Network for Treatment of AIDS (PENTA) 5 Trial. *Journal of Infectious Diseases.* 2002;186(3):312-320.
22. Lisse IM, Aaby P, Whittle H, Jensen H, Engelmann M, Christensen LB. T-lymphocyte subsets in West African children: impact of age, sex, and season. *Journal of Pediatrics.* 1997;130(1):77-85.
23. Embree J, Bwayo J, Nagelkerke N, et al. Lymphocyte subsets in human immunodeficiency virus type 1-infected and uninfected children in Nairobi. *Pediatric Infectious Disease Journal.* 2001;20(4):397-403.
24. Shearer WT, Rosenblatt HM, Gelman RS, et al. Lymphocyte subsets in healthy children from birth through 18 years of age: the Pediatric AIDS Clinical Trials Group P1009 study. *Journal of Allergy and Clinical Immunology.* 2003;112(5):973-980.
25. Patel K, Hernan MA, Williams PL, et al. Long-term effects of highly active antiretroviral therapy on CD4+ cell evolution among children and adolescents infected with HIV: 5 years and counting. *Clinical Infectious Diseases.* 2008;46(11):1751-1760.
26. Yin DE, Warshaw MG, Miller WC, et al. Using CD4 percentage and age to optimize pediatric antiretroviral therapy initiation. *Pediatrics.* 2014;134(4):e1104-1116.
27. Salazar-Vizcaya L, Keiser O, Karl T, et al. Viral load versus CD4(+) monitoring and 5-year outcomes of antiretroviral therapy in HIV-positive children in Southern Africa: a cohort-based modelling study. *AIDS.* 2014;28(16):2451-2460.
28. NASCOP K. Guidelines for Antiretroviral Therapy in Kenya. 4 ed. Nairobi, Kenya: Ministry of Health; 2011.
29. Tucker JD, Bien CH, Easterbrook PJ, et al. Optimal strategies for monitoring response to antiretroviral therapy in HIV-infected adults, adolescents, children and pregnant women: a systematic review. *AIDS.* 2014;28 Suppl 2:S151-160.
30. Rutherford GW, Anglemyer A, Easterbrook PJ, et al. Predicting treatment failure in adults and children on antiretroviral therapy: a systematic review of the performance characteristics of the 2010 WHO immunologic and clinical criteria for virologic failure. *AIDS.* 2014;28 Suppl 2:S161-169.
31. Rawizza HE, Chaplin B, Meloni ST, et al. Immunologic criteria are poor predictors of virologic outcome: implications for HIV treatment monitoring in resource-limited settings. *Clinical Infectious Diseases.* 2011;53(12):1283-1290.
32. Violari A, Cotton MF, Gibb DM, et al. Early antiretroviral therapy and mortality among HIV-infected infants. *New England Journal of Medicine.* 2008;359(21):2233-2244.

33. Innes S, Lazarus E, Otwombe K, et al. Early severe HIV disease precedes early antiretroviral therapy in infants: Are we too late? *Journal of the International AIDS Society.* 2014;17:18914.
34. Wamalwa D, Benki-Nugent S, Langat A, et al. Survival benefit of early infant antiretroviral therapy is compromised when diagnosis is delayed. *Pediatric Infectious Disease Journal.* 2012;31(7):729-731.
35. Wamalwa DC, Farquhar C, Obimbo EM, et al. Medication diaries do not improve outcomes with highly active antiretroviral therapy in Kenyan children: a randomized clinical trial. *Journal of the International AIDS Society.* 2009;12:8.
36. NASCOP K. Guidelines to Antiretroviral Therapy in Kenya. 2nd ed. Nairobi, Kenya: Ministry of Health; 2003.
37. NASCOP K. Guidelins for Antiretroviral Therapy in Kenya 3rd ed. Nairobi, Kenya: Ministry of Health; 2005.
38. Emery S, Bodrug S, Richardson BA, et al. Evaluation of performance of the Gen-Probe human immunodeficiency virus type 1 viral load assay using primary subtype A, C, and D isolates from Kenya. *Journal of Clinical Microbiology.* 2000;38(7):2688-2695.
39. Huenecke S, Behl M, Fadler C, et al. Age-matched lymphocyte subpopulation reference values in childhood and adolescence: application of exponential regression analysis. *European Journal of Haematology.* 2008;80(6):532-539.
40. Penazzato M, Prendergast AJ, Muhe LM, Tindyebwa D, Abrams EJ. Optimization of antiretroviral therapy in HIV-infected children under 3 years of age: a systematic review. *AIDS.* 2014;28 Suppl 2:S137-146.
41. Wiznia A, Stanley K, Krogstad P, et al. Combination nucleoside analog reverse transcriptase inhibitor(s) plus nevirapine, nelfinavir, or ritonavir in stable antiretroviral therapy-experienced HIV-infected children: week 24 results of a randomized controlled trial--PACTG 377. Pediatric AIDS Clinical Trials Group 377 Study Team. *AIDS Res Hum Retroviruses.* 2000;16(12):1113-1121.
42. Wamalwa DC, Lehman DA, Benki-Nugent S, et al. Long-term virologic response and genotypic resistance mutations in HIV-1 infected Kenyan children on combination antiretroviral therapy. *Journal of Acquired Immune Deficiency Syndromes.* 2013;62(3):267-274.
43. Vreeman RC, Wiehe SE, Pearce EC, Nyandiko WM. A systematic review of pediatric adherence to antiretroviral therapy in low- and middle-income countries. *Pediatric Infectious Disease Journal.* 2008;27(8):686-691.
44. Turkova A, Webb RH, Lyall H. When to start, what to start and other treatment controversies in pediatric HIV infection. *Paediatric Drugs.* 2012;14(6):361-376.
45. Lodha R, Manglani M. Antiretroviral therapy in children: recent advances. *Indian Journal of Pediatrics.* 2012;79(12):1625-1633.
46. Gogtay JA, Malhotra G. Reformulation of existing antiretroviral drugs. *Current Opinion in HIV and AIDS.* 2013;8(6):550-555.
47. Prendergast A, Mphatswe W, Tudor-Williams G, et al. Early virological suppression with three-class antiretroviral therapy in HIV-infected African infants. *AIDS.* 2008;22(11):1333-1343.
48. Kuhn L, Abrams EJ, Palumbo P, et al. Maternal versus paternal inheritance of HLA class I alleles among HIV-infected children: consequences for clinical disease progression. *AIDS.* 2004;18(9):1281-1289.
49. Rosenblatt HM, Stanley KE, Song LY, et al. Immunological response to highly active antiretroviral therapy in children with clinically stable HIV-1 infection. *Journal of Infectious Diseases.* 2005;192(3):445-455.

50. Puthanakit T, Kerr S, Ananworanich J, Bunupuradah T, Boonrak P, Sirisanthana V. Pattern and predictors of immunologic recovery in human immunodeficiency virus-infected children receiving non-nucleoside reverse transcriptase inhibitor-based highly active antiretroviral therapy. *Pediatric Infectious Disease Journal.* 2009;28(6):488-492.
51. Vrisekoop N, van Gent R, de Boer AB, et al. Restoration of the CD4 T cell compartment after long-term highly active antiretroviral therapy without phenotypical signs of accelerated immunological aging. *Journal of Immunology.* 2008;181(2):1573-1581.
52. Chiappini E, Galli L, Zazzi M, de Martino M. Immunological recovery despite virological failure is independent of human immunodeficiency virus-type 1 resistant mutants in children receiving highly active antiretroviral therapy. *Journal of Medical Virology.* 2003;70(4):506-512.
53. Marconi VC, Grandits G, Okulicz JF, et al. Cumulative viral load and virologic decay patterns after antiretroviral therapy in HIV-infected subjects influence CD4 recovery and AIDS. *PloS One.* 2011;6(5):e17956.
54. Prendergast A, Tudor-Williams G, Jeena P, Burchett S, Goulder P. International perspectives, progress, and future challenges of paediatric HIV infection. *Lancet.* 2007;370(9581):68-80.
55. Peacock-Villada E, Richardson BA, John-Stewart GC. Post-HAART outcomes in pediatric populations: comparison of resource-limited and developed countries. *Pediatrics.* 2011;127(2):e423-441.
56. Koller M, Patel K, Chi BH, et al. Immunodeficiency in children starting antiretroviral therapy in low-, middle-, and high-income countries. *Journal of Acquired Immune Deficiency Syndromes.* 2015;68(1):62-72.
57. Paiardini M, Muller-Trutwin M. HIV-associated chronic immune activation. *Immunological Reviews.* 2013;254(1):78-101.
58. Haas A, Zimmermann K, Oxenius A. Antigen-dependent and -independent mechanisms of T and B cell hyperactivation during chronic HIV-1 infection. *Journal of Virology.* 2011;85(23):12102-12113.
59. Hazenberg MD, Otto SA, van Benthem BH, et al. Persistent immune activation in HIV-1 infection is associated with progression to AIDS. *AIDS.* 2003;17(13):1881-1888.
60. Deeks SG, Kitchen CM, Liu L, et al. Immune activation set point during early HIV infection predicts subsequent CD4+ T-cell changes independent of viral load. *Blood.* 2004;104(4):942-947.
61. Douek DC, Roederer M, Koup RA. Emerging concepts in the immunopathogenesis of AIDS. *Annual Review of Medicine.* 2009;60:471-484.
62. Giorgi JV, Hultin LE, McKeating JA, et al. Shorter survival in advanced human immunodeficiency virus type 1 infection is more closely associated with T lymphocyte activation than with plasma virus burden or virus chemokine coreceptor usage. *Journal of Infectious Diseases.* 1999;179(4):859-870.
63. Plaeger-Marshall S, Isacescu V, O'Rourke S, Bertolli J, Bryson YJ, Stiehm ER. T cell activation in pediatric AIDS pathogenesis: three-color immunophenotyping. *Clinical Immunology and Immunopathology.* 1994;71(1):19-26.
64. Schlesinger M, Peters V, Jiang JD, Roboz JP, Bekesi JG. Increased expression of activation markers on CD8 lymphocytes in children with human immunodeficiency virus-1 infection. *Pediatric Research.* 1995;38(3):390-396.
65. Pilakka-Kanthikeel S, Kris A, Selvaraj A, Swaminathan S, Pahwa S. Immune activation is associated with increased gut microbial translocation in treatment-naive, HIV-infected children in a resource-limited setting. *Journal of Acquired Immune Deficiency Syndromes.* 2014;66(1):16-24.

66. Gallagher K, Gorre M, Harawa N, et al. Timing of lymphocyte activation in neonates infected with human immunodeficiency virus. *Clinical and Diagnostic Laboratory Immunology.* 1997;4(6):742-747.
67. Ruel T, Ssewanyana I, Achan J, et al. Dynamics of T cell activation accompanying CD4 recovery in antiretroviral treated HIV-infected Ugandan children. *Clinical Immunology.* 2009;131(3):410-414.
68. Ssewanyana I, Baker CA, Ruel T, et al. The Distribution and Immune Profile of T Cell Subsets in HIV-Infected Children from Uganda. *AIDS Research and Human Retroviruses.* 2009;25(1):65-71.
69. Romeiro JR, Pinto JA, Silva ML, Eloi-Santos SM. Further evidence that the expression of CD38 and HLA-DR(+) in CD8(+) lymphocytes does not correlate to disease progression in HIV-1 vertically infected children. *Journal of the International Association of Physicians in AIDS Care (Chicago, Ill. : 2002).* 2012;11(3):164-168.
70. Freguja R, Gianesin K, Mosconi I, et al. Regulatory T cells and chronic immune activation in human immunodeficiency virus 1 (HIV-1)-infected children. *Clinical and Experimental Immunology.* 2011;164(3):373-380.
71. Resino S, Bellon JM, Gurbindo MD, Munoz-Fernandez MA. CD38 expression in CD8+ T cells predicts virological failure in HIV type 1-infected children receiving antiretroviral therapy. *Clinical Infectious Diseases.* 2004;38(3):412-417.
72. Vigano A, Saresella M, Rusconi S, Ferrante P, Clerici M. Expression of CD38 on CD8 T cells predicts maintenance of high viraemia in HAART-treated HIV-1-infected children. Highly active antiretroviral therapy. *Lancet.* 1998;352(9144):1905-1906.
73. Sherman GG, Scott LE, Galpin JS, et al. CD38 expression on CD8(+) T cells as a prognostic marker in vertically HIV-infected pediatric patients. *Pediatric Research.* 2002;51(6):740-745.
74. De Rosa SC, Thomas EP, Bui J, et al. HIV-DNA priming alters T cell responses to HIV-adenovirus vaccine even when responses to DNA are undetectable. *Journal of Immunology.* 2011;187(6):3391-3401.
75. Ssewanyana I, Elrefaei M, Dorsey G, et al. Profile of T cell immune responses in HIV-infected children from Uganda. *Journal of Infectious Diseases.* 2007;196(11):1667-1670.
76. Paul ME, Shearer WT, Kozinetz CA, Lewis DE. Comparison of CD8(+) T-cell subsets in HIV-infected rapid progressor children versus non--rapid progressor children. *Journal of Allergy and Clinical Immunology.* 2001;108(2):258-264.
77. Slyker JA, Lohman-Payne BL, John-Stewart GC, et al. Acute cytomegalovirus infection in Kenyan HIV-infected infants. *AIDS.* 2009;23(16):2173-2181.
78. Kourtis AP, Ibegbu CC, Wiener J, et al. Role of intestinal mucosal integrity in HIV transmission to infants through breast-feeding: the BAN study. *Journal of Infectious Diseases.* 2013;208(4):653-661.
79. Bello G, Velasco-de-Castro CA, Bongertz V, et al. Immune activation and antibody responses in non-progressing elite controller individuals infected with HIV-1. *Journal of Medical Virology.* 2009;81(10):1681-1690.
80. Sauce D, Almeida JR, Larsen M, et al. PD-1 expression on human CD8 T cells depends on both state of differentiation and activation status. *AIDS.* 2007;21(15):2005-2013.
81. Holm M, Pettersen FO, Kvale D. PD-1 predicts CD4 loss rate in chronic HIV-1 infection better than HIV RNA and CD38 but not in cryopreserved samples. *Curr HIV Res.* 2008;6(1):49-58.
82. Papagno L, Spina CA, Marchant A, et al. Immune activation and CD8+ T-cell differentiation towards senescence in HIV-1 infection. *PLoS Biology.* 2004;2(2):E20.

83. Hulstaert F, Hannet I, Deneys V, et al. Age-related changes in human blood lymphocyte subpopulations. II. Varying kinetics of percentage and absolute count measurements. *Clinical Immunology and Immunopathology.* 1994;70(2):152-158.
84. de Martino M, Rossi ME, Azzari C, Gelli MG, Galli L, Vierucci A. Different meaning of CD38 molecule expression on CD4+ and CD8+ cells of children perinatally infected with human immunodeficiency virus type 1 infection surviving longer than five years. *Pediatric Research.* 1998;43(6):752-758.
85. Paul ME, Mao C, Charurat M, et al. Predictors of immunologic long-term nonprogression in HIV-infected children: implications for initiating therapy. *Journal of Allergy and Clinical Immunology.* 2005;115(4):848-855.
86. Giorgi JV, Ho HN, Hirji K, et al. CD8+ lymphocyte activation at human immunodeficiency virus type 1 seroconversion: development of HLA-DR+ CD38- CD8+ cells is associated with subsequent stable CD4+ cell levels. The Multicenter AIDS Cohort Study Group. *Journal of Infectious Diseases.* 1994;170(4):775-781.
87. Ferbas J, Kaplan AH, Hausner MA, et al. Virus burden in long-term survivors of human immunodeficiency virus (HIV) infection is a determinant of anti-HIV CD8+ lymphocyte activity. *Journal of Infectious Diseases.* 1995;172(2):329-339.
88. Hua S, Lecuroux C, Saez-Cirion A, et al. Potential role for HIV-specific CD38-/HLA-DR+ CD8+ T cells in viral suppression and cytotoxicity in HIV controllers. *PloS One.* 2014;9(7):e101920.
89. Saez-Cirion A, Lacabaratz C, Lambotte O, et al. HIV controllers exhibit potent CD8 T cell capacity to suppress HIV infection ex vivo and peculiar cytotoxic T lymphocyte activation phenotype. *Proceedings of the National Academy of Sciences of the United States of America.* 2007;104(16):6776-6781.
90. Saez-Cirion A, Sinet M, Shin SY, et al. Heterogeneity in HIV suppression by CD8 T cells from HIV controllers: association with Gag-specific CD8 T cell responses. *Journal of Immunology.* 2009;182(12):7828-7837.
91. Hunt PW, Cao HL, Muzoora C, et al. Impact of CD8+ T-cell activation on CD4+ T-cell recovery and mortality in HIV-infected Ugandans initiating antiretroviral therapy. *AIDS.* 2011;25(17):2123-2131.
92. Goicoechea M, Smith DM, Liu L, et al. Determinants of CD4+ T cell recovery during suppressive antiretroviral therapy: association of immune activation, T cell maturation markers, and cellular HIV-1 DNA. *Journal of Infectious Diseases.* 2006;194(1):29-37.
93. McCloskey TW, Kohn N, Lesser M, Bakshi S, Pahwa S. Immunophenotypic analysis of HIV-infected children: alterations within the first year of life, changes with disease progression, and longitudinal analyses of lymphocyte subsets. *Cytometry.* 2001;46(3):157-165.
94. Rainwater-Lovett K, Nkamba H, Mubiana-Mbewe M, Moore CB, Margolick J, Moss WJ. Changes in cellular immune activation and memory T-cell subsets in HIV-infected Zambian children receiving HAART. *Journal of Acquired Immune Deficiency Syndromes.* 2014;67(5):455-462.
95. Sousa AE, Carneiro J, Meier-Schellersheim M, Grossman Z, Victorino RM. CD4 T cell depletion is linked directly to immune activation in the pathogenesis of HIV-1 and HIV-2 but only indirectly to the viral load. *Journal of Immunology.* 2002;169(6):3400-3406.
96. Resino S, Galan I, Bellon JM, Navarro ML, Leon JA, Munoz-Fernandez MA. Characterizing the immune system after long-term undetectable viral load in HIV-1-infected children. *Journal of Clinical Immunology.* 2003;23(4):279-289.
97. Margolick JB, Gange SJ, Detels R, O'Gorman MR, Rinaldo CR, Jr., Lai S. Impact of inversion of the CD4/CD8 ratio on the natural history of HIV-1 infection. *Journal of Acquired Immune Deficiency Syndromes.* 2006;42(5):620-626.

98. Bäumler CB, Böhler T, Herr I, Benner A, Krammer PH, Debatin KM. Activation of the CD95 (APO-1/Fas) system in T cells from human immunodeficiency virus type-1-infected children. *Blood.* 1996;88(5):1741-1746.
99. Slyker JA, Rowland-Jones SL, Dong T, et al. Acute cytomegalovirus infection is associated with increased frequencies of activated and apoptosis-vulnerable T cells in HIV-1-infected infants. *Journal of Virology.* 2012;86(20):11373-11379.
100. Grayson JM, Zajac AJ, Altman JD, Ahmed R. Cutting edge: increased expression of Bcl-2 in antigen-specific memory CD8+ T cells. *Journal of Immunology.* 2000;164(8):3950-3954.
101. Trautmann L, Janbazian L, Chomont N, et al. Upregulation of PD-1 expression on HIV-specific CD8+ T cells leads to reversible immune dysfunction. *Nature Medicine.* 2006;12(10):1198-1202.
102. Ladell K, Hellerstein MK, Cesar D, Busch R, Boban D, McCune JM. Central memory CD8+ T cells appear to have a shorter lifespan and reduced abundance as a function of HIV disease progression. *Journal of Immunology.* 2008;180(12):7907-7918.
103. Montesano C, Anselmi A, Palma P, et al. HIV replication leads to skewed maturation of CD8-positive T-cell responses in infected children. *New Microbiologica.* 2010;33(4):303-309.
104. Paiardini M, Cervasi B, Albrecht H, et al. Loss of CD127 expression defines an expansion of effector CD8+ T cells in HIV-infected individuals. *Journal of Immunology.* 2005;174(5):2900-2909.
105. Okoye AA, Picker LJ. CD4(+) T-cell depletion in HIV infection: mechanisms of immunological failure. *Immunological Reviews.* 2013;254(1):54-64.
106. Corbeau P, Reynes J. Immune reconstitution under antiretroviral therapy: the new challenge in HIV-1 infection. *Blood.* 2011;117(21):5582-5590.
107. Rigaud M, Borkowsky W, Muresan P, et al. Impaired immunity to recall antigens and neoantigens in severely immunocompromised children and adolescents during the first year of effective highly active antiretroviral therapy. *Journal of Infectious Diseases.* 2008;198(8):1123-1130.
108. Resino S, Seoane E, Perez A, Ruiz-Mateos E, Leal M, Munoz-Fernandez MA. Different profiles of immune reconstitution in children and adults with HIV-infection after highly active antiretroviral therapy. *BMC Infectious Diseases.* 2006;6:112.
109. Rainwater-Lovett K, Moss WJ. Immunologic basis for revaccination of HIV-infected children receiving HAART. *Future Virology.* 2011;6(1):59-71.
110. Vigano A, Vella S, Saresella M, et al. Early immune reconstitution after potent antiretroviral therapy in HIV-infected children correlates with the increase in thymus volume. *AIDS.* 2000;14(3):251-261.
111. Gibb DM, Newberry A, Klein N, de Rossi A, Grosch-Woerner I, Babiker A. Immune repopulation after HAART in previously untreated HIV-1-infected children. Paediatric European Network for Treatment of AIDS (PENTA) Steering Committee. *Lancet.* 2000;355(9212):1331-1332.
112. Sutcliffe CG, Moss WJ. Do children infected with HIV receiving HAART need to be revaccinated? *Lancet Infectious Diseases.* 2010;10(9):630-642.
113. Boulware DR, Hullsiek KH, Puronen CE, et al. Higher levels of CRP, D-dimer, IL-6, and hyaluronic acid before initiation of antiretroviral therapy (ART) are associated with increased risk of AIDS or death. *Journal of Infectious Diseases.* 2011;203(11):1637-1646.
114. Schacker TW, Reilly C, Beilman GJ, et al. Amount of lymphatic tissue fibrosis in HIV infection predicts magnitude of HAART-associated change in peripheral CD4 cell count. *AIDS.* 2005;19(18):2169-2171.

115. Cheret A, Bacchus-Souffan C, Avettand-Fenoel V, et al. Combined ART started during acute HIV infection protects central memory CD4+ T cells and can induce remission. *Journal of Antimicrobial Chemotherapy.* 2015;70(7):2108-2120.
116. Elrefaei M, McElroy MD, Preas CP, et al. Central memory CD4+ T cell responses in chronic HIV infection are not restored by antiretroviral therapy. *Journal of Immunology.* 2004;173(3):2184-2189.
117. Sagnia B, Ateba Ndongo F, Ndiang Moyo Tetang S, et al. Reference values of lymphocyte subsets in healthy, HIV-negative children in Cameroon. *Clinical and Vaccine Immunology.* 2011;18(5):790-795.
118. Lunn PG. The impact of infection and nutrition on gut function and growth in childhood. *Proceedings of the Nutrition Society.* 2000;59(1):147-154.
119. Hua W, Jiao Y, Zhang H, et al. Central memory CD4 cells are an early indicator of immune reconstitution in HIV/AIDS patients with anti-retroviral treatment. *Immunological Investigations.* 2012;41(1):1-14.
120. Tinago W, Coghlan E, Macken A, et al. Clinical, immunological and treatment-related factors associated with normalised CD4+/CD8+ T-cell ratio: effect of naive and memory T-cell subsets. *PloS One.* 2014;9(5):e97011.

VITA

Kristjana Ásbjörnsdóttir was born in Akureyri, Iceland and grew up in Iceland and Malawi. She received a BSc in Biochemistry from the University of Iceland in 2005 and moved to the United States in 2007, when she began working at Stanford University. Kristjana received her MPH and PhD in Epidemiology from the University of Washington in 2012 and 2015, respectively.