ACCOUNTING FOR ADHERENCE IN COMPARISONS OF PEDIATRIC ANTIRETROVIRAL THERAPY

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ABSTRACT

Dwight Edward Yin: Accounting for Adherence in Comparisons of Pediatric Antiretroviral Therapy
(Under the direction of Stephen R. Cole)

Near-perfect adherence to antiretroviral therapy (ART) is necessary to prevent treatment failure and resistance**.** In children, protease-inhibitor (PI)-based regimens appear more potent than non-nucleoside reverse transcriptase inhibitor (NNRTI)-based regimens, but poor PI taste and tolerability may lead to worse adherence. We aimed to disentangle relationships among ART regimen, adherence, and viral control.

In Aim 1, we assessed treatment-naïve children for differences in time to treatment disruption across randomly assigned PI versus NNRTI-based initial ART. At 4 years, the hazard ratio (HR) for treatment disruptions on PI versus NNRTI-based regimens was 1.19 (95% confidence interval [CI] 0.88-1.61). By study end, treatment disruption probabilities converged with HR 1.11 (95% CI 0.84-1.48). Reported reasons for treatment disruptions suggested that PI participants experienced greater tolerability problems.

In Aim 2, we estimated the per-protocol effect of initial PI versus NNRTI-based ART on time to treatment failure in children with HIV in a setting of ideal adherence. After an intention-to-treat (ITT) analysis, we generated per-protocol estimates by administratively right-censoring participants experiencing protocol deviations (non-medically indicated treatment disruption or dropout) and using inverse-probability of censoring weights to correct for imparted informative censoring. In ITT analysis, PI participants experienced 4-year treatment failure probabilities of 41.3% versus 39.5% (NNRTIs), risk difference (RD) 1.8%, HR 1.09 (95% CI 0.74-1.60). In per-

protocol analysis, PI participants experienced treatment failure probabilities of 35.5% versus 29.5% (NNRTI), RD 6.4%, HR 1.30 (95% CI 0.80-2.12). Protocol deviations were non-differential across arms. Shifts in failure probabilities from the ITT to per-protocol analysis were 5.7% (PI) versus 10.3% (NNRTI).

In conclusion, children experienced similar time to treatment disruption for initial PI- and NNRTI-based ART, despite greater PI tolerability problems. With ideal adherence, NNRTIs appeared more potent, but PIs appeared more robust against non-adherence. Principal drivers of the observed parent study null effects were not non-adherence, but rather regimen potency and robustness. Shifts in ITT to per-protocol estimates yield a novel method of quantifying regimen robustness. We conclude that regimen potency and robustness, in addition to traditional components (*e.g.*, adherence), provide a more detailed framework for the various elements contributing to the composite outcome of treatment efficacy.

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LIST OF ABBREVIATIONS

AIDS	Acquired Immunodeficiency Syndrome
ART	Antiretroviral therapy
CI	Confidence interval
DHHS	Department of Health and Human Services
HIV	Human Immunodeficiency Virus
HR	Hazard ratio
IMPAACT	International Maternal Pediatric Adolescent AIDS Clinical Trials Group
INSTI	Integrase strand transferase inhibitor
IPCW	Inverse probability of censoring weights
ITT	Intention-to-treat analysis
LPV/r	Lopinavir with boosting-dose ritonavir
MEMS	Medication Event Monitoring System
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NRTI	Nucleoside/nucleotide reverse transcriptase inhibitors
PACTG	Pediatric AIDS Clinical Trials Group
PENTA	Paediatric European Network for Treatment of AIDS
PI	Protease inhibitor
PMTCT	Prevention of mother-to-child transmission
RD	Risk difference
US	United States
WHO	World Health Organization

CHAPTER 1: INTRODUCTION

A. The Burden of HIV in Pediatric Populations

Pediatric HIV-related mortality is high, but mortality is decreasing with widespread antiretroviral therapy (ART). Globally, 1.8 million children under age 15 years are living with HIV.[1] This number continues to increase due to better survival and approximately 180,000 newly infected children each year. Untreated, mortality by age 18 months in HIV-infected children is as high as 51%.[2] Although ART has improved pediatric HIV long-term survival,[3] HIV/AIDS still kills 110,000 children annually.[1] To attain maximum and sustained decreases in pediatric HIV mortality, we must optimize pediatric ART.

B. Infants, Children, and Adolescents with HIV are Vulnerable Populations

Infants, children, and adolescents suffer greater consequences from ART treatment failure. First, due to a lack of studies, greater variability in drug absorption and pharmacokinetics, and age-related changes, children have fewer licensed antiretroviral drugs.[4,5] Even some licensed drugs are becoming more limited in utility, as antiretroviral use in prevention of mother-to-child transmission (PMTCT) of HIV has selected for antiretroviral resistance.[6-14]

Second, long-term toxicity and tolerability have greater implications in children.[15] Pediatric populations must face longer potential treatment duration, as therapy started during infancy may lead to drug exposure on the order of 70 years. Due to greater cumulative drug exposure and potential impact on growth, children are more vulnerable than adults to metabolic side effects of therapy, including lipodystrophy, dyslipidemia, insulin resistance,

hyperlactactemia, osteopenia, and growth failure.[16,17] Greater cumulative drug exposure is also associated with increased cardiovascular risk, which may be a potential long-term complication as children progress into adulthood.[16] Progression to secondary or salvage regimens typically means more toxic regimens. Both drug toxicity and intolerance, especially poor palatability, have compromised pediatric ART adherence.[18-20]

Third, ART appears to be less successful in producing viral suppression in children,[21] who may require more potent regimens to achieve viral suppression.[5] Adolescents have particularly worse viral and immunological outcomes due to poor ART adherence.[22-25]

Finally, children are more prone to acquisition of antiretroviral resistance mutations due to: (1) higher plasma viral loads, allowing more spontaneous mutations; (2) less robust antiviral immune responses; (3) pharmacokinetic challenges with concomitant requirements for more frequent dosing; and (4) social and behavioral dependency.[5]

Thus, treatment failure has great consequences in children.

C. The Critical Role of Adherence to ART

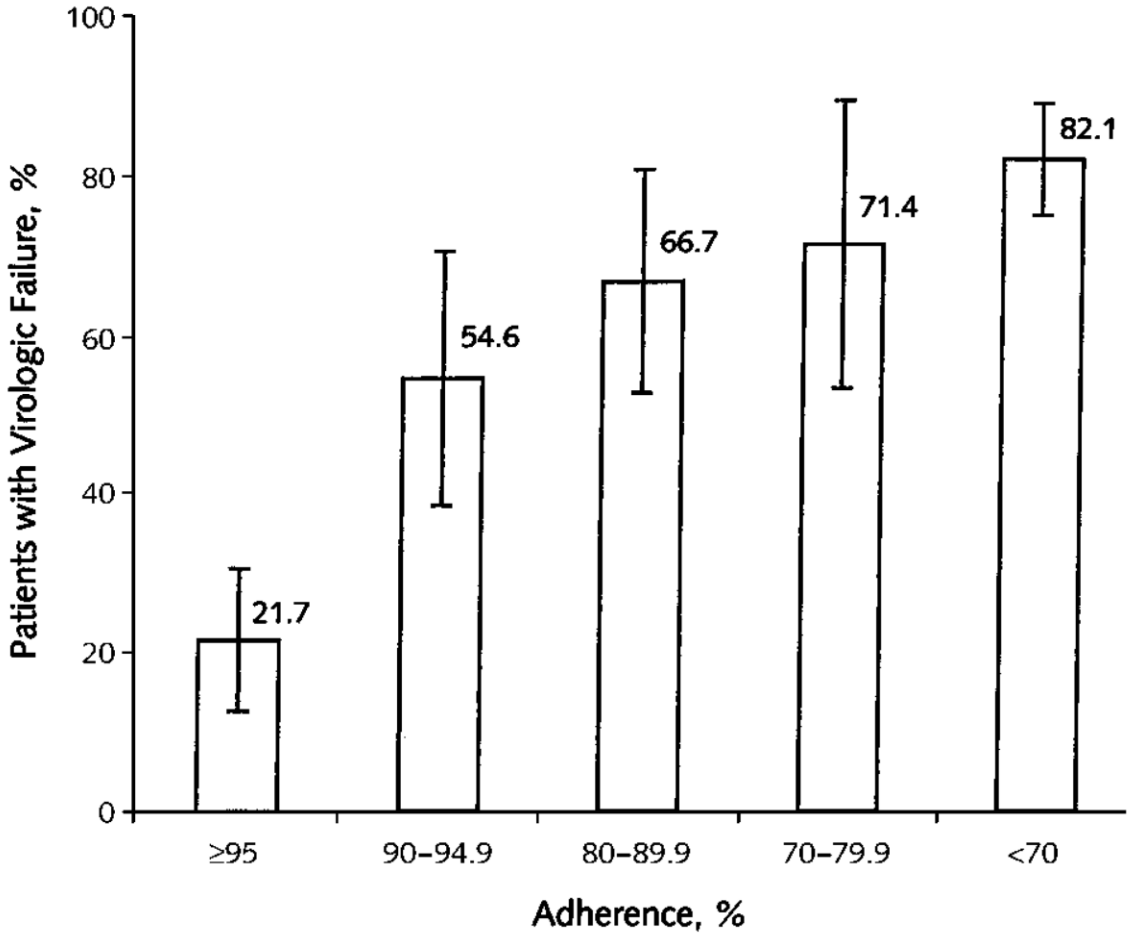
Although HIV viral replication can be suppressed by ART, virions arise from multiple compartments.[26] Unfortunately, HIV establishes a stable form of latency as integrated provirus in resting memory T cells and macrophages.[27,28] While only a small fraction (<0.05%) of resting CD4+ T cell population in the circulation or lymph nodes carries integrated HIV-1 DNA and only an even smaller fraction are replication-competent, the estimated total body number of resting CD4+ T cells with integrated HIV-1 DNA ranges from 4.6×10^7 to 3.4×10^7 cells.[28] Even with prolonged suppression of plasma viremia on ART, replication-competent virus can be routinely recovered from resting CD4+ T cells,[29,30] and infrequent detection of new drug resistance mutations provide evidence for viral latency rather than drug failure.[30] As memory CD4+ T cells survive for months to years,[31] ART must sustain viral suppression indefinitely or

risk rebound of viral replication.

Optimal and sustained outcomes are strongly associated with the degree of treatment adherence.[22,23,32,33] The sentinel data in adults found that a mere 10% decrease in adherence was associated with a doubling of HIV RNA viral load.[32,34] Paterson *et. al.* were the first to demonstrate the minimal room for error in adherence. In HIV-infected adults, 99 patients were prescribed HAART with an unboosted protease inhibitor and followed for a median of 6 months (range, 3 to 15 months) using adherence measured by a Medication Event Monitoring System (MEMS). At their last study visit, patients with ≥95% adherence had the lowest proportion of viral failure (viral load >400 copies/mL; Figure 1.1). Even slightly worse adherence at 90 to 94.9% increased viral failure by 33%. Subjects with ≥95% adherence also had better CD4+ lymphocyte counts, fewer days in the hospital, and no new opportunistic infections or deaths.[33]

The adherence effect on decreasing viral load also decreases the probability of HIV sub-populations acquiring antiretroviral resistance mutations,[5,35] preserving the longevity of current and future ART regimens. Unfortunately, initial studies of adult ART adherence using objective measures estimated average adherence around 70%,[36-39] especially among those who failed to achieve viral suppression.[40]

Figure 1.1. Relationship between adherence to ART and viral failure. From *Annals of Internal Medicine*, Paterson DL, Swindells S, Mohr J, et al. Adherence to protease inhibitor therapy and outcomes in patients with HIV infection, Vol. 133, Issue 1, Page 24. Copyright © 2000 American College of Physicians. All Rights Reserved. Reprinted with the permission of American College of Physicians, Inc.[33]



D. Drug-Adherence Relationships in Children

Choice of antiretroviral drugs may affect adherence. Drug characteristics can make pediatric adherence more difficult.[19,20,41-44] The greatest contrast in commonly used pediatric drugs is between non-nucleoside reverse transcriptase inhibitors (NNRTI) and protease inhibitors (PI).

PIs have higher drug toxicity, especially gastrointestinal side effects, and intolerance,

particularly their noxious taste.[18-20] Although a highly potent PI, lopinavir with boosting doses of ritonavir (LPV/r), has better viral suppression than NNRTIs,[41] a recent clinical trial failed to identify better treatment success in children randomized to PIs than NNRTIs[45]—perhaps due to differences in adherence.

Antiretroviral drugs also differ in packaging, such as availability in liquid or pill formulations.[46,47] Even when children are able to swallow pills, certain protease inhibitors are only available as large pills, complicating swallowing for children. No protease inhibitors are available as complete-regimen combinations, whereas combined drugs with NNRTIs can facilitate adherence through administration of fewer pills.[4]

Increased dosing frequency has been associated with worse adherence,[18,48-50] whereas some NNRTIs, most notably efavirenz, have more suitable pharmacokinetics for once daily dosing administration. Thus, NNRTIs offer many adherence advantages of PIs.

Buscher et. al. performed a prospective observational cohort study of 99 ART-naïve patients, comparing ART regimen and dosing frequency using a visual analogue scale to measure adherence. Participants taking once-daily regimens had modestly higher adherence (99.5%), compared to twice-daily regimens (94% adherence). However, once daily fixed dose combinations (100% adherence) were similar to regimens of two or more pills (99.3% adherence). Thus, dose frequency but not pill burden was associated with adherence.[51]

However, in a Maggiolo *et. al.* study of adults, both dose frequency and pill burden were associated with adherence. In this study, NNRTI-based therapy was more acceptable than PI-based therapy.[52]

E. Adherence-Response Relationships Differ by Drug Class

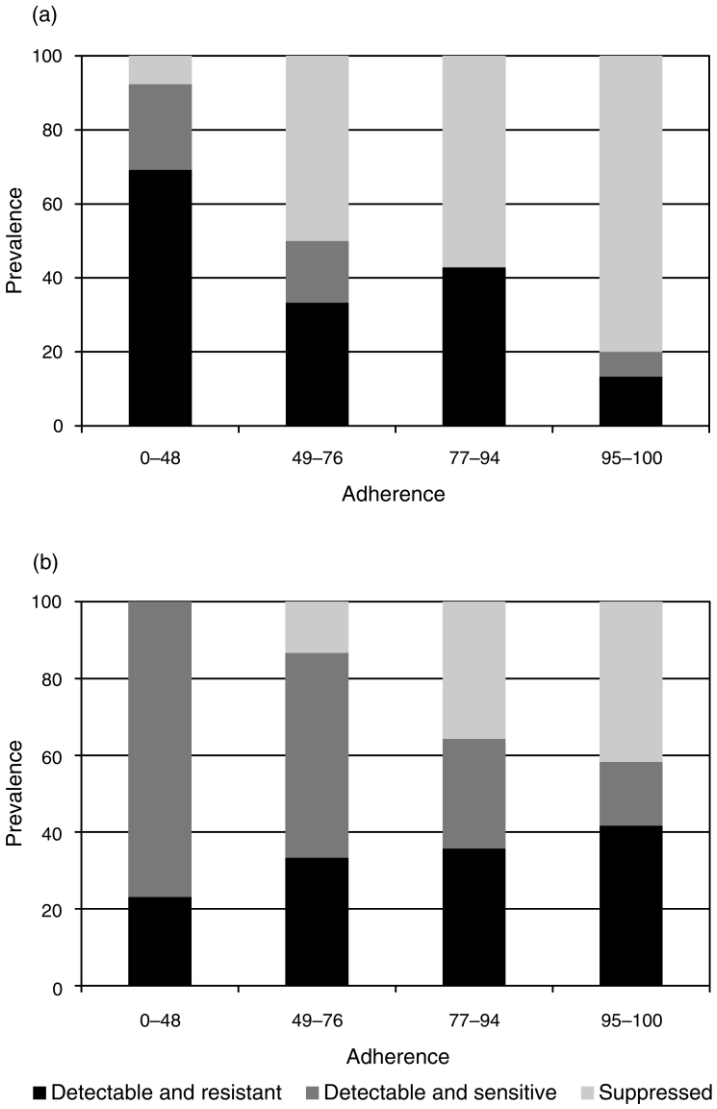
Although these early studies first defined minimum ART adherence targets for unboosted PI regimens, subsequent studies suggested that adherence targets should vary by drug class.

Much of these subsequent data have focused on the relationship between adherence and drug resistance—related to drug potency, viral fitness, adherence patterns, and pharmacogenomics.[53,54]

Bangsberg *et. al.* evaluated the prevalence of resistance by adherence levels in patients treated with NNRTIs or unboosted PIs, using unannounced pill count measures for adherence, viral load monitoring, genotypic resistance testing, and single-cycle recombinant phenotypic susceptibility assays to test replication capacity. NNRTI-based regimens were associated with better viral suppression to <50 copies/mL, but in patients failing to suppress, NNRTI regimens also had more frequent resistance, which was related to viral fitness. At 0-48% adherence, 69% of the NNRTI group had resistance, while 23% of the PI group had resistance (Figure 1.2).[55]

Another study by Bangsberg found similar results in a cohort of 110 participants on NNRTI or unboosted PI therapy. Participants were followed for a median 9.1 months with monthly adherence measurements by unannounced pill counts or MEMS and monthly viral load measurements. The majority of NNRTI-treated participants were suppressed to <400 copies/mL at adherence 54-100%, which was much more common than unboosted PI-treated participants, particularly in the 53-74% adherence stratum.[39]

Figure 1.2. Prevalence of viral suppression, viral failure without resistance, and viral failure with resistance by adherence quartile and drug regimen. (a) Patients treated with non-nucleoside reverse transcriptase inhibitor; (b) patients treated with protease inhibitor. Reprinted from Bangsberg DR, Acosta EP, Gupta R, et al., Adherence-resistance relationships for protease and non-nucleoside reverse transcriptase inhibitors explained by virological fitness, *AIDS*, Vol. 20, Issue 2, Page 227, Copyright © 2006 Lippincott Williams & Wilkins, with permission from Wolters Kluwer Health, Inc.[55]



Maggiolo *et. al*.'s cross-sectional study of 543 patients had more viral suppression in patients receiving NNRTI-based ART over PI-based ART at similar levels of adherence, as measured by an adherence questionnaire.[52] Better viral suppression was noted primarily at

adherence of 75-95%. This study compared NNRTIs to grouped data on patients receiving boosted or unboosted PI regimens. However, participants had different levels of treatment experience and regimens spanned first line therapy to salvage therapy.

A subsequent study by Maggiolo *et. al.* of a cohort including 1,133 participants on stable NNRTI or PI ART compared NNRTI ART to separate categories of unboosted and boosted PI ART. NNRTI ART had more viral suppression than either PI group at adherence <95%.[56]

In contrast, some adult and pediatric data indicate that boosted PIs are more forgiving of suboptimal adherence than unboosted PIs or NNRTIs.[57-59] Similarly, a cohort study of 78 children in South Africa compared a boosted PI (ritonavir-boosted lopinavir) with unboosted PI and NNRTI ART.[60] Adherence was measured by MEMS, and viral loads were measured every 6 months. Children who were receiving PI-based regimens had more viral suppression, even with poor treatment adherence. Notably, the majority of patients were exposed to single-dose nevirapine for PMTCT, which may have predisposed these children to higher risk of NNRTI failure, and ten children were on second-line therapy.

A major randomized pediatric trial comparing PI-based to NNRTI-based ART in treatment-naïve children without single-dose nevirapine exposure is the PENPACT-1 (PENTA 9/PACTG 390) study. This study identified no significant differences in viral failure by treatment regimen.[45] The adherence analysis of PENPACT-1 is the subject of this proposal.

F. Measuring Adherence and Treatment Disruptions

The PENPACT-1 trial measured adherence using the Pediatric AIDS Clinical Trials Group (PACTG) adherence questionnaires (Appendices 1 and 2). The PACTG adherence questionnaires were first validated by Van Dyke *et. al.*[61] The validation study was performed in a cohort of 193 children aged 4 months to 17 years in a 4-arm study including various 3- or 4-drug combinations of stavudine, lamivudine, nevirapine, ritonavir, and nelfinavir enrolled between

December 1997 and September 1998. Questionnaires were administered every 3 months and relied on the number of doses of each medication missed during the 3 days preceding the study visit. Responses were categorized into full adherence and non-full adherence, defined as missing no doses and missing at least one dose, respectively. Seventy percent of children reported full adherence, and 30% of children reported non-full adherence. Adherence was reported as 68% for nelfinavir but about 80% for all other drugs. Challenges to adherence were reported most frequently for ritonavir and nelfinavir, including poor taste, patient refusal, and scheduling problems. Adherence was associated with viral response. Full adherence was reported in 92% of children with a $\geq 2 \log_{10}$ drop in viral load, but full adherence was reported in only 64% of children with $<2 \log_{10}$ drop in viral load.

Unfortunately, adherence questionnaires cannot capture all aspects of adherence. Not only are questionnaires prone to measurement error in self-reporting, but they may not capture patterns of adherence. Patterns of missed doses may have differential effects on viral control. For boosted protease inhibitors, average adherence is the primary predictor of viral suppression. Parenti *et. al.* evaluated two cohorts with a total of 72 patients who were prescribed ritonavir-boosted PI regimens and compared patterns of missed doses. Average adherence was a stronger predictor of viral suppression than duration or frequency of treatment interruptions.[62] In contrast for NNRTIs, a cohort study of 71 adult patients on nevirapine- or efavirenz-based ART identified repeated unplanned treatment interruption of ≥48 hours as an independent predictor of viral failure and resistance to the NNRTI class.[63]

G. Moving from Adherence to Treatment Disruptions and Protocol Deviations

More recent advances in the analysis of pragmatic clinical trials have moved from traditional adherence analyses to estimating per-protocol effect estimates.[64] These per-protocol effect estimates generate estimates that represent treatment effectiveness when the treatment is

used as indicated by the protocol, *i.e.* what would have been observed if all patients had adhered to the trial protocol. The result is an estimate that adjusts for post-randomization factors, including confounding bias due to incomplete adherence or use of off-protocol concomitant therapies and selection bias due to differential loss to follow-up.

In general, these per-protocol estimates of pragmatic trials have relied on censoring participants for non-medically indicated reasons, censoring when it is no longer certain that participants are receiving treatment, and adjusting for bias due to incomplete adherence. Thus, of interest is no longer merely adherence to a specific prescription, but rather adherence to a protocol, which may allow some tolerance for non-adherence.

This method overcomes historical challenges to per-protocol analyses. The primary origin of objections to traditional per-protocol analyses have been the results of the Coronary Drug Project. In this study, the original per-protocol analysis generated a 9.4% risk difference in 5-year mortality between adherers and non-adherers to placebo.[65] Although this original analysis generated skepticism on the validity of traditional per-protocol analyses, developments in analytical methods since that time, such as g-methods, have generated more plausible risk differences of 2.5%, ranging from -0.7% to 4.5%, using the more recent per-protocol methods, thereby supporting the validity of this approach.[66,67] Such methods have also been applied to adult ART initiation studies, substantiating the increasing acceptance of this approach.[68,69]

As a result, estimating adherence to a protocol is now the more relevant analytical approach and comprises a composite of traditional adherence measures, use of off-protocol medications, and loss to follow-up. Such a definition of protocol adherence may be more aptly called treatment disruptions or protocol deviations. As a result, below we refer to protocol adherence and refer to violations of protocol adherence as either treatment disruptions or

protocol deviations, thereby making “non-adherence”, “treatment disruptions”, and “protocol deviations” generally interchangeable terms that will be used throughout the remainder of this document.

H. Critical Review of the Literature

To date, publications discussing drug-related factors on adherence have strong limitations. First, no pediatric drug-specific adherence studies have randomized patients to their comparison drug regimens, leaving potential unmeasured confounding, including confounding by indication. Second, most studies have been cross-sectional, failing to account for changes in adherence over time and generating prevalent associations, rather than incident associations. Third, these studies fail to account for the cyclical relationship between adherence and viral control, namely that adherence affects viral control, which affects interventions taken to address adherence. Fourth, most studies have been restricted in their size and distribution of their study populations, often taking place at one or a few centers in one country, thus potentially limiting their generalizability. Fifth, many studies do not account for the measurement error in their adherence assessments, which can vary greatly according to the measurement method[19,70-73]: physician perception,[71,74] self-report or caregiver report,[24,46,71,73,75,76] structured questionnaires,[19,72] pharmacy refill data,[19,70,71] appointments kept,[70,71] medication measurement systems (*e.g.* pill counts, visual analogue scales for syrups),[71,73,77] medication event monitoring systems,[78-81] or therapeutic drug monitoring.[82,83] Finally, few studies have accounted for protocol non-adherence by applying more recent developments in estimating the per-protocol effect.[84] These study deficiencies limit the ability to generate inferences regarding relationships among ART regimen, adherence, and viral control.

CHAPTER 2: STATEMENT OF SPECIFIC AIMS

A. Overview

For HIV-infected children, optimal survival outcomes can only be achieved with near-perfect antiretroviral therapy (ART) adherence. Adherence lapses result in treatment failure and acquisition of resistance mutations, which are particularly consequential in children: Compared with adults, children have fewer available licensed drugs; greater pharmacokinetic variability; more potential for toxicity from longer lifetime drug exposure; greater social vulnerability; and more rapid progression to HIV disease and death.[20,43,47,50,85-87] Poor adherence may also jeopardize the validity of studies that estimate the outcomes of different treatment regimens.[88] Few pediatric studies have evaluated longitudinal relationships among ART regimens, adherence, and viral control. These relationships become entangled in clinical trials comparing different ART regimens, potentially biasing results. Therefore, applying causal inference methods may disentangle relationships among ART regimen, adherence, and viral control, allowing (1) identification of ART regimens that pose greater adherence challenges and (2) more valid estimation of ART treatment efficacy with good adherence.

Unfortunately, prior pediatric studies on the ART regimens, adherence, and viral control have methodological limitations. First, no prior pediatric adherence studies have used data on children that underwent randomized allocation; thereby leaving these studies open to residual confounding from unmeasured covariates. Secondly, pediatric studies on the relationship between ART regimen and viral control have failed to account for the time-varying nature of adherence, limiting analyses to either cross-sectional designs or intent-to-treat analyses. These

shortcomings limit the ability to generate inferences about relationships among ART regimen, adherence, and viral control.

In the PENPACT-1 study, 266 HIV-1-infected, treatment-naïve children from Europe, North America, and South America were randomized to ART with either a PI or NNRTI.[45] No significant differences were identified in viral control over 4 years of ART. We suspect that these results may be explained by opposing effects of adherence and regimen potency.

Our overarching hypothesis is that in HIV-infected children initiating ART, PI-based therapy (vs. NNRTI-based therapy) will have worse adherence that is compensated by better viral control.

B. Aim 1

We aim to assess PENPACT-1 participants for differences in time to first treatment disruption across randomized PI vs. NNRTI treatment arms at 4 years and end of study. Using existing data on 266 HIV-1-infected, ART-naïve children randomized to initial ART with a PI or NNRTI, we will compare time to treatment disruption longitudinally over 4 years and end of study, derived from ART regimen stops or switches on treatment records and 24-weekly questionnaires of reported missed doses within 3 days of clinic visit. We will further explore reasons for missed doses by documented reasons for treatment stops or switches and reported reasons for missed doses within 14 days of clinic visit as reported on adherence questionnaires. We will use Kaplan-Meier estimators and Cox proportional hazards models to estimate the risk of treatment disruption at 4 years after randomization and at the end of study (*i.e.*, 6 years).

Hypothesis: We hypothesize that participants receiving initial PI-based ART will report worse adherence. We further hypothesize that reasons for treatment disruption will demonstrate worse tolerability for PI regimens.

Rationale: To optimize clinical outcomes, clinicians must consider both drug

pharmacology and adherence to ART regimen. In children, the poor taste and gastrointestinal toxicity of PIs may lead to worse adherence.[18-20,41-43] Even if children are able to swallow pills, certain PIs are available only as large pills.[89] No PIs are available as complete-regimen combinations for children, whereas single-tablet NNRTI regimens can facilitate adherence through administration of fewer pills.[4,90-93] Higher dosing frequency has also been associated with more frequent treatment disruptions.[48,51,90,92] Some NNRTIs, most notably efavirenz, have more suitable pharmacokinetics for once daily administration, whereas most PI-based regimens are administered at least twice daily.[94] In sum, the noxious taste, worse toxicity and tolerability, unavailability of single-tablet regimen, and more frequent administration requirements of PIs found our hypothesis of worse expected adherence in the PI arm.

C. Aim 2

We aim to estimate the per-protocol effect of initial PI versus NNRTI-based ART on time to treatment failure in treatment-naïve children living with HIV in developed countries in a setting of ideal adherence. First, we will generate time to treatment disruption under conventional intention-to-treat (ITT) analysis. Then, we will use Robins-Finkelstein inverse probability-of-censoring weights to correct for differences in protocol non-adherence by PI vs. NNRTI regimen.[88,95] Adherence correction will allow estimation of viral control by PI vs. NNRTI regimen under conditions of good adherence throughout the 4-year follow-up period. We will then compare the shift in ITT to per-protocol estimates of PI vs. NNRTI initial regimens on time to viral failure.

Hypothesis: We hypothesize that with good adherence, PI regimens will have better viral control. We hypothesize that under the ITT analysis allowing post-randomization protocol deviations, initial PI and NNRTI-based ART will have similar time to viral control. We further hypothesize that under optimal adherence, initial PI therapy will be more robust against protocol

deviations, with less of a shift in ITT versus per-protocol estimates in time to viral failure.

Rationale: The ITT parameter estimates the effect of being assigned to a treatment protocol. In standard ITT analysis of clinical trials, poor adherence typically biases estimates of effective treatments towards the null, resulting in under-estimation of treatment efficacy and over-estimation of treatment safety and tolerability. The per-protocol parameter estimates the effect of being assigned to, and remaining on, a treatment protocol. The Robins-Finkelstein (RF) inverse-probability of censoring weights (IPCW) method of per-protocol analysis provides a principled way to address problematic post-randomization protocol violations, such as poor adherence and study dropout, which may affect clinical trial results.[95]

In PENPACT-1, we suspect that the null result of the ITT analysis may have resulted from a cancellation effect: Worse adherence to PIs cancelled their more robust viral efficacy. As described in Aim 1 above, we hypothesize that PI regimens will have worse adherence than NNRTI regimens. However, in Aim 2 we suspect that PIs will be more robust against protocol deviations. Boosted PI-based regimens appear more forgiving of treatment disruptions than NNRTI-based regimens.[60,96-98] In a pediatric trial comparing continuing a boosted PI regimen to switching to an NNRTI, inadequate adherence had less influence on viral outcomes in the boosted PI arm.[99] Another pediatric trial of a boosted PI versus NNRTI regimen found greater viral efficacy for the boosted PI.[100] Thus, we expect that PIs will be more robust against protocol deviations in this study, thereby cancelling any identified non-adherence effects.

D. Conclusion

By combining these two aims, we plan to disentagle effects of pediatric ART regimens and adherence on HIV viral control, thereby allowing clinicians to consider optimal regimens that affect both adherence and viral control.

CHAPTER 3: METHODS

A. Overview

We plan to disentangle relationships among drug regimen, protocol non-adherence, and HIV viral control in HIV-infected pediatric populations—a population with high risk for high mortality and long-term consequences of treatment failure. Disentangling these relationships will occur via two steps presented in two aims. In Aim 1, we will first estimate differences in treatment disruption between randomized protease inhibitor (PI) versus non-nucleoside reverse transcriptase inhibitor (NNRTI) ART regimens. In Aim 2, we will estimate viral suppression by ART regimen under traditional ITT analysis, then under a per-protocol analysis accounting for protocol deviation. This result will illustrate the shift in effect estimates when allowing protocol deviations (ITT analysis) and when correcting for protocol non-adherence (per-protocol analysis). With increased knowledge about the inter-relationships among drug regimen, protocol adherence, and viral control, clinicians would be able to make more informed decisions about ART regimens to use in infants, children, and adolescents, including patients at high risk for poor ART adherence.

The methods we will apply will help overcome common limitations in the published literature by implementing the following novel elements:

- *One of the first randomized controlled trial on pediatric drug regimen adherence:* Most prior work on drug-related pediatric ART adherence has been observational, allowing for possible unmeasured confounding, which would be mitigated by treatment randomization.

- *Collaboration of two international pediatric HIV research networks:* This proposed work is the first collaboration between the two major international pediatric HIV clinical trials groups: Pediatric AIDS Clinical Trials Group (PACTG)—renamed International Maternal Pediatric Adolescent AIDS Clinical Trials Group (IMPAACT)—and Paediatric European Network for Treatment of AIDS (PENTA). This collaboration results in a broadly representative study population, involving 68 study sites in 13 countries in North America, South America, and Europe.
- *Longitudinal analysis of adherence and viral control:* We will perform longitudinal analysis of relationships among PI versus NNRTI regimen, protocol adherence, and viral control over 4 years. We will assess the changing adherence patterns over time and how adherence relates to changing viral control over time. This approach will allow disentagling of cyclical relationships among time-varying adherence, which leads to changes in viral control, which leads to interventions to improve adherence, which again affects adherence.

These methodological elements relate to study design, study population, and statistical analysis. Study design and population will be discussed in Section B as context for both Aim 1 and Aim 2. Statistical methods will differ by aim and are described separately for Aim 1 (Section C) or Aim 2 (Section D).

B. Study Design and Population

B1. Overview of Study Design

We will analyze data collected from PENPACT-1 (PACTG 390 / PENTA 9), an international multicenter phase 2/3, randomized, open-label trial enrolling children infected with HIV-1 from 68 clinical centers in Europe and North and South America between September 25, 2002, and September 7, 2005.[45] At entry, children were simultaneously assigned (1:1): (a) to start

ART with two nucleoside reverse transcriptase inhibitors (NRTI) plus either a PI or an NNRTI and also (b) to switch from first-line to second-line ART at viral-load thresholds of either 1,000 copies/mL or 30,000 copies/mL. The treating clinician chose the two NRTI drugs combined with a drug from the randomly assigned PI or NNRTI class.

Children underwent clinical and HIV-1 RNA viral load assessments at randomization (week 0), weeks 2, 4, 8, 12, 16, 24, and then every 12 weeks until the last child assigned to treatment reached 4 years of follow-up (August 31, 2009). Treatment starts, changes, and stoppages were recorded at clinical visits and *ad hoc* throughout the study, along with reasons for the medication change. Trained study personnel administered validated adherence questionnaires every 24 weeks after randomization. Adherence questionnaires recorded the number of missed doses to all antiretrovirals over the 3 days prior to visit and barriers to adherence experienced within 2 weeks prior to visit.

Aim 1 will compare PI versus NNRTI treatment arms longitudinally over 4 years and end of study for differences in time to treatment disruption, as measured by study treatment records and 24-weekly adherence questionnaires. **Aim 2** will estimate the effect of initial PI versus NNRTI ART on time to treatment failure under a traditional ITT analysis, then under a per-protocol analysis correcting for protocol non-adherence over 4 years.

B2. Study Population

The study population will include the 263 participants (of 266 randomized) from the ITT analysis of PENPACT-1. Eligible children were >30 days and <18 years of age with a confirmed age-appropriate HIV laboratory diagnosis on two separate positive peripheral blood specimens from different days. The subject had to be antiretroviral-naïve or have received less than 56 consecutive days after birth of antiviral drugs used to prevent mother-to-infant transmission of HIV.

Participants were excluded from PENPACT-1 if the participant had any of the following: infant or maternal peripartum exposure to nevirapine for prevention of mother-to-child HIV transmission; current grade 3 or 4 clinical or laboratory toxicity; an active opportunistic infection and/or serious bacterial infection at study entry; contraindications to receiving the trial therapies; receipt of any cytotoxic therapy for malignancy; pregnancy or breastfeeding.

The characteristics of PENPACT-1's ITT study population are listed in Table 3.1. After excluding three participants who did not start ART, a total of 263 were included from the following countries: United States 75, Germany 21, Spain 2, France 17, Italy 22, Romania 31, Brazil 41, United Kingdom 37, Austria 2, Bahamas 4, and Argentina 11. We expect this study population to be representative of newly diagnosed HIV-infected children receiving care at HIV centers in industrialized countries.

Table 3.1. Baseline characteristics of participants in the modified intention-to-treat analysis of PENPACT-1.

		Randomized Group		
Variable		PI	NNRTI	Total
N		131	132	263
Age				
<3 years	*n* (%)	34 (26%)	36 (27%)	70 (27%)
3-17 years	*n* (%)	97 (74%)	96 (73%)	193 (73%)
Age in years	Median (IQR)	7.1 (2.8, 13.7)	6.4 (2.7, 11.0)	6.5 (2.8, 12.9)
Sex				
Male	*n* (%)	69 (53%)	67 (51%)	136 (52%)
Race				
Black, Non-Hispanic	*n* (%)	60 (46%)	69 (52%)	129 (49%)
White, Non-Hispanic	*n* (%)	40 (31%)	29 (22%)	69 (26%)
Hispanic/Other	*n* (%)	31 (24%)	34 (26%)	65 (25%)
Research Network[a]				
PENTA	*n* (%)	95 (73%)	93 (70%)	188 (71%)
PACTG/IMPAACT	*n* (%)	36 (27%)	39 (30%)	75 (29%)
Route of Infection				
Vertical	*n* (%)	103 (79%)	106 (80%)	209 (79%)
Other/Unknown	*n* (%)	28 (21%)	26 (20%)	54 (21%)
CDC Clinical Stage				
N	*n* (%)	27 (21%)	29 (22%)	56 (21%)
A	*n* (%)	35 (27%)	37 (28%)	72 (27%)
B	*n* (%)	41 (31%)	43 (33%)	84 (32%)
C	*n* (%)	28 (21%)	23 (17%)	51 (19%)
Weight-for-Age Z-score	Median (IQR)	-0.5 (-1.6, 0.1)	-0.7 (-1.6, 0.2)	-0.6 (-1.6, 0.1)
Height-for-Age Z-score	Median (IQR)	-0.9 (-1.5, -0.2)	-0.9 (-1.8, 0)	-0.9 (-1.7, -0.2)
CD4 Z score	Median (IQR)	-3.6 (-7.2, -1.7)	-3.4 (-6.5, -1.4)	-3.5 (-6.8, -1.6)
Viral Load $\log_{10}$ copies/mL	Median (IQR)	5.1 (4.5, 5.7)	5.0 (4.5, 5.6)	5.0 (4.5, 5.7)
Perinatal ART Exposure	*n* (%)	19 (15%)	20 (15%)	39 (15%)
≥1 Major Resistance Mutation[b]	*n/N* (%)	5/116 (4%)	5/123 (4%)	10/239 (4%)
HIV-1 subtype				
B	*n* (%)	52 (42%)	49 (39%)	101 (41%)
C	*n* (%)	13 (11%)	12 (10%)	25 (10%)
F	*n* (%)	25 (20%)	23 (18%)	48 (19%)
A/CRF_AG/D/G	*n* (%)	21 (17%)	31 (25%)	52 (21%)
Unclassified	*n* (%)	12 (10%)	11 (9%)	23 (9%)
Switching Threshold				
1,000 copies/mL	*n* (%)	66 (50%)	68 (52%)	134 (51%)
30,000 copies/mL	*n* (%)	65 (50%)	64 (48%)	129 (49%)

Abbreviations: ART, antiretroviral therapy; IQR, interquartile range; *N*, total sample size; *n*, subsample size; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor.

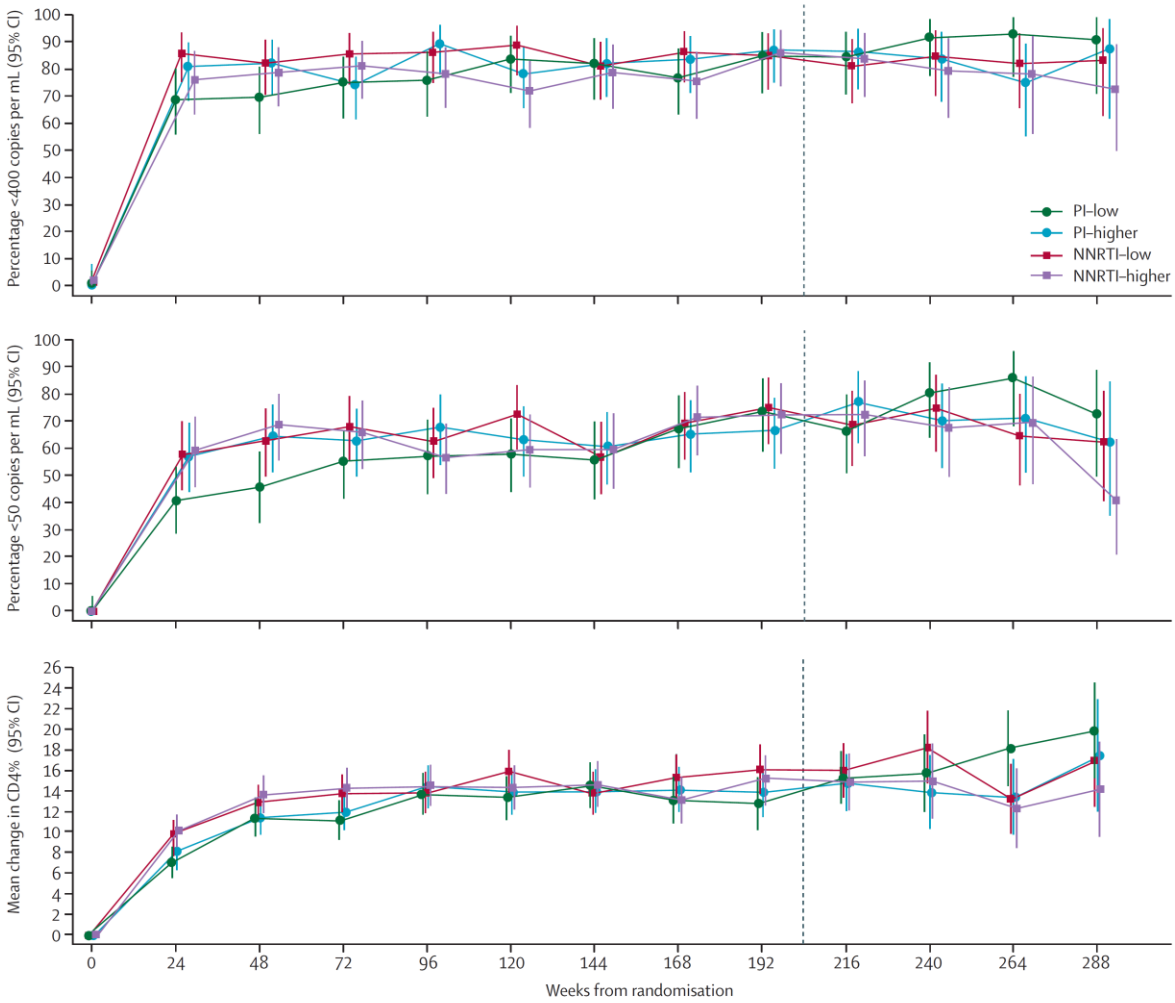
[a] PENTA sites were predominantly in Europe, South America, and the Bahamas. PACTG/IMPAACT sites were based primarily in the United States.

[b] Not all patients had successful baseline genotypic resistance assays.

B3. Parent Study Results

In PENPACT-1, 234 of 263 (89%) of enrollees were in follow-up at 4 years, the primary endpoint. The results of the parent study found little difference between PIs and NNRTIS in (a) mean changes in viral load from baseline to 4 years, which were -3.31 $\log_{10}$ copies/mL for protease inhibitors versus -3.31 $\log_{10}$ copies/mL for NNRTIs [difference, -0.15 $\log_{10}$ copies/ml; 95% CI, -0.41 to 0.11] or (b) proportion of children with viral loads <400 copies/mL at 4 years, which were 82% for PIs versus 82% for NNRTIs [OR 0.97; 95% CI, 0.49-1.91; Figure 3.1].[45] Given prior data on PIs being more efficacious than NNRTIs in lowering viral load,[41] we hypothesize that this lack of difference in viral suppression between PIs and NNRTIs was due to worse adherence to PIs, yet greater efficacy of PIs in poor adherence.

Figure 3.1. Viral suppression and CD4 percentage changes during follow-up in the PENPACT-1 trial. Vertical line indicates 4 years after randomization, the primary endpoint. CD4%: CD4 percentage; NNRTI: non-nucleoside reverse transcriptase inhibitor regimen; PI: protease inhibitor regimen; higher: higher viral load switch threshold (30,000 copies per mL); low: lower switch threshold (1,000 copies per mL). Reprinted from *Lancet Infectious Diseases*, Vol. 11, PENPACT-1 (PENTA 9/PACTG 390) Study Team, Babiker A, Castro nee Green H, et al., First-line antiretroviral therapy with a protease inhibitor versus non-nucleoside reverse transcriptase inhibitor and switch at higher versus low viral load in HIV-infected children: an open-label, randomised phase 2/3 trial, Page 277, Copyright (2011), with permission from Elsevier.[45]



C. Aim 1: Assess PENPACT-1 Participants for Differences in Time to Treatment Disruption Across Randomized PI vs. NNRTI Treatment Arms at 4 Years and End of Study

C1. Study Design

We will perform a secondary analysis of data from a randomized, controlled trial (PENPACT-1) comparing pediatric ART with PIs versus NNRTIs for their effects on time to treatment disruption at the parent study's primary endpoint of 4 years and end of study.

C2. Treatment Assessment

The parent study randomized 266 participants to PIs vs. NNRTIs, of which 263 were started on ART. Randomization was stratified by age (<3 years or ≥3 years), region (PACTG or PENTA centers), and exposure to perinatal ART to reduce mother-to-child transmission. Computer-generated sequentially numbered randomization lists (with variable block sizes) were prepared by the trial statistician (who was not involved in regimen allocation) and securely incorporated within the PACTG or PENTA databases, which allowed access to the next number but not the whole list. This study was an open-label study with no masking.

This analysis will be a modified ITT analysis, excluding three of 266 participants (1%) who never started ART and four (2%) that were treated contrary to randomization. The primary analysis will explore whether the regimen contained either a PI or an NNRTI, regardless of other drugs in the regimen. PIs will include lopinavir, nelfinavir, ritonavir, saquinavir, indinavir, amprenavir, fosamprenavir, atazanavir, tipranavir, and darunavir. PIs given concomitantly to low-dose ritonavir will be defined as boosted PI regimens, whereas PIs not given concomitantly to low-dose ritonavir will be defined as unboosted PI regimens. NNRTI regimens will include nevirapine, efavirenz, delaviridine, and etravirine. Treatment groups will be classified according to randomized 1st line regimen, PI versus NNRTI. Treatment categorization will begin at the date of randomization.

C3. Outcome Assessment

We will define time to treatment disruption as the number of weeks between randomization and the first documented treatment disruption event. We will derive treatment disruption events from the trial treatment records and adherence questionnaires. We will define treatment disruption as stopping, switching, or reporting missed doses of any component of the initial ART regimen for any reason other than systematic drug recalls or planned treatment interruptions.

Treatment record events were recorded at clinical visits and *ad hoc* throughout the study. Participant treatment records recorded all treatment events, including ART starts, stops, temporary suspensions, restarts, drug substitutions, regimens switches, line of ART regimen, and reasons for the treatment events. Treatment disruptions derived from the treatment record will include ART stoppages (including stops and temporary suspensions) or switches (including drug substitutions and regimen switches).

Trained study personnel administered validated adherence questionnaires every 24 weeks after randomization. Adherence questionnaires recorded the number of missed doses to all antiretrovirals over the 3 days prior to visit and barriers to adherence experienced within 2 weeks prior to visit. Treatment disruptions derived from the questionnaire will include any questionnaire-reported missed doses within 3 days prior to the study visit.

If the participant had assumed responsibility for his/her own drug regimen, the questionnaire was administered to the participant (Appendices II and III). The questionnaire's adherence measures will use answers to the following questions:

- <u>Primary Question:</u> Over the last 3 days, can you say how many times, (*sic*) you have missed a dose? (The response options include a global "no missed drugs" option or a response itemizing individual drugs and numbers of doses missed in each of the past 3

days.)

- Secondary Question:
    - If you have missed any doses during the last two weeks, please tick the reason(s).

If the participant had not assumed responsibility for his/her own drug regimen, the questionnaire was administered to the participant's primary caregiver, *i.e.*, the person responsible for administering the prescribed drugs at home (Appendices II and III). In this case, the questions to be used for adherence measures are:

- Primary Question: Over the last 3 days, can you say how many times, (*sic*) your child has missed a dose? (The response options include a global "no missed drugs" option or a response itemizing individual drugs and numbers of doses missed in each of the past 3 days.)
- Secondary Question:
    - If your child has missed any doses during the last two weeks, please indicate the reason(s) and say which drug(s).

Any acknowledgement of missed doses in 3 days or 2 weeks will be defined as poor, as consistent with the initial validation study.[61] Validity of binary categorization, rather than more detailed response levels, will be explored with categorical analysis.

Adherence questionnaires were standardized within according to research networks (PENTA vs. PACTG), and the protocol teams worked to harmonize the questionnaires across networks (Appendices II and III). Questions regarding number of missed doses over the 3 days prior to clinic visit were consistent across network questionnaires, as were the 14-day periods of assessments for reasons for non-adherence. However, differences in choices listed for reasons for non-adherence, listed tools used to support adherence, and protocol specific regarding who

administered or answered the questionnaires may result in heterogeneity of responses across networks, ages, and respondents regarding these specifics.

As a result, questionnaire components of outcomes will focus on the more reliable questionnaire measures. For the primary outcome, time to treatment disruption will include the questionnaire inquiry regarding missed doses over the prior 3 days. For secondary outcomes, we explore the reasons listed for missed doses of the prior 2 weeks.

C4. Covariate Assessment

Since we will be analyzing treatments across randomized arms using an ITT analysis, we will rely on randomization to average across differences in treatment arms. However, randomization was stratified on age (<3 vs. 3-17 years), exposure to perinatal antiretrovirals (yes vs. no), and research network/region (PACTG/IMPAACT vs. PENTA). Thus, the randomization protocol introduced a stratified data structure, for which we will account in additional analyses, using these stratification variables as covariates.

C5. Statistical Analyses

PI vs. NNRTI treatment groups will be assessed according to a modified ITT analysis consistent with the original study. The sole modification will be removal of three participants: two who withdrew consent prior to ART initiation, and one with a major eligibility violation. Follow-up will begin at date of randomization. Participants will be right-censored for initial treatment contrary to randomization, planned treatment interruption, death, withdrawal of consent, loss to follow-up, or study end.

Additional analyses will include adjustment for stratified randomization factors (age, receipt of perinatal ART prophylaxis, research network), assess differences in outcome for the primary follow-up time point (4 years) vs. the entire study, and explore reasons for treatment disruptions. Reasons for treatment disruptions will be analyzed using (1) the treatment record's

documented rationale for ART stop or change and (2) any questionnaire-reported barriers to adherence within 2 weeks prior to the visit when missed dose(s) were reported.

We will also assess the sensitivity of our results to our definition of treatment disruption. Our alternative outcome definitions will include: restricting treatment record treatment disruptions to drug changes or stops lasting more than 3 days or 14 days, restricting treatment record treatment disruptions to only events including the PI or NNRTI drug component, including two consecutive missing adherence questionnaires (plus a 6-week lag for a late visit) as a treatment disruption, and right-censoring after two consecutive missing adherence questionnaires.

For the primary outcome, we will estimate the risk of treatment disruptions using the complement of the Kaplan-Meier estimator. We will estimate the hazard ratio for treatment disruptions using Cox proportional hazards models. Proportional hazards assumptions will be assessed graphically, using time-interaction terms, and with martingale residuals. In adjusted analyses, we will stratify by baseline randomized stratification variables: age, exposure to perinatal ART, and research network (which varied by region).

Analyses will be conducted in SAS® version 9.4 (Cary, NC).

C6. Limitations

Our principal limitation is expected to be measurement error. First, we have no direct measures of drug exposure, such as therapeutic drug monitoring. Treatment records can only capture prescribing events and documented ART disruptions, and the adherence questionnaires rely on accurate reporting by either the child or the caregiver, if present and willing to answer. Second, missing questionnaires or refusal to answer portions of the adherence questionnaire may bias results. Third, adherence questionnaires in this study focus on ART adherence over the 3 days prior to the most recent visit and inquire about adherence barriers encountered over the

prior 2 weeks, rather than a daily measure of adherence throughout the study. The time-varying nature of treatment disruption means that participants may experience an initial or temporary period of treatment disruption that is subsequently corrected, but our analysis will present only data on time to the first event of treatment disruption. Finally, heterogeneity of adherence questionnaires across networks, ages, and respondents regarding reasons for treatment disruption may limit interpretability of these responses despite efforts to harmonize these questionnaires.

C7. Addressing Limitations

To mitigate the potential information bias from reporting biases and missing data, we are designing our study to evaluate a composite outcome. Our combining treatment records and adherence questionnaires into a composite outcome should decrease measurement error from either instrument individually.

Secondly, we are using a previously validated pediatric adherence questionnaire. Responses to this questionnaire were associated with concurrent viral suppression and decline in viral load from baseline (Table 3.2), which varied by week of study.[61] Differences in reported adherence were associated with the questionnaire respondent (child, biological parent, other) and medication formulation (liquid vs. tablet/powder). The questionnaire analysis relied on missed doses over the prior 3 days, which serves as the basis for our approach. This question has been the most validated of the questionnaire and the most commonly used in the research literature.[101] However, performance characteristics of this questionnaire have not been validated against a gold standard measure of adherence.

Table 3.2. Associations with full adherence (FA) reported on questionnaire. Reproduced with permission from *Pediatrics*, Vol. 109, Page 4, Copyright © 2002 by the AAP.[61]

Variable	Rate of FA
Number of patients	125
HIV RNA copy number (copies/mL)*	
<400	77% (58/75)
400–800	63% (10/16)
>800	55% (17/31)
HIV RNA reduction from baseline ($\log_{10}$ copies/mL)†	
<2 $\log_{10}$	64% (63/98)
≥2 $\log_{10}$	92% (22/24)
Responder to adherence questionnaire‡	
Child	47% (7/15)
Biological parent	65% (22/34)
Other	78% (59/76)
Protease inhibitor formulation§	
Liquid	88% (22/25)
Tablet or powder	65% (62/96)

* Wilcoxon exact $P = .02$.
† Fisher exact $P = .01$.
‡ Fisher exact $P = .04$.
§ Fisher exact $P = .03$.

Thirdly, we have performed an analysis to confirm that the questionnaires have predictive value for viral control in the PENPACT-1 data. Linear mixed effects models evaluating the relationship between the adherence questionnaire (missed doses in past 3 days) and viral load suggest that this questionnaire performs at least reasonably well as a measure of adherence over the first 24 weeks of ART, when ART resistance is less likely (Table 3.3, Appendix IV Figure S1). Additionally, the adherence questionnaire response proportion was high (91%), without significantly differential response according to drug regimen ($P = 0.87$). Thus, we expect to obtain reliable results from Aim 1.

Table 3.3. Differences in decline of HIV RNA log10 copies/mL decline over 24 weeks of ART by adherence category.

Questionnaire-Reported Adherence	Decline of HIV RNA $\log_{10}$ copies/mL	Difference in rate of decline (95% CI)	*P* value
Good	-1.83	-0.70	< .0001
Poor	-1.13	(-0.37, -1.03)	

Missing data from questionnaire nonresponse may bias results. To assess whether the data may be missing at random, we have assessed potential relationships between baseline variables and missing questionnaires. Although race may have some association with missingness, most variables do not appear strongly associated (Table 3.4).

Table 3.4. Baseline characteristics of 263 HIV-1-infected, treatment-naïve children initiated on 1st line ART with distribution of missing values.

Variable		Good Adherence	Poor Adherence	Missing	Total	Missingness *P* value
Age at ART[a] Initiation	n (%)	49 (92)	4 (4)	14	67 (100)	0.45
0 to <3 years	n (%)	43 (96)	2 (4)	12	57 (100)	
3 to <6 years	n (%)	59 (91)	6 (9)	9	4 (100)	
6 to <13 years	n (%)	41 (77)	12 (23)	12	65 (100)	
13 to <18 years						
Sex						
Male	n (%)	99 (88)	13 (12)	24	136 (100)	1.00
Female	n (%)	93 (89)	11 (11)	23	127 (100)	
Race						
Black	n (%)	92 (83)	19 (17)	18	129 (100)	0.11
Non-Black	n (%)	100 (95)	5 (5)	29	134 (100)	
ART Regimen						
Protease Inhibitor	n (%)	92 (86)	15 (14)	15	131 (100)	0.87
NNRTI[a]	n (%)	100 (92)	9 (8)	9	132 (100)	
Viral Load Threshold for ART Switch						
≥1,000 copies/mL	n (%)	97 (88)	13 (12)	24	134	1.00
≥30,000 copies/mL	n (%)	95 (90)	11 (10)	23	129	
Baseline Viral Load Log_{10} copies/mL	Mean (SD)	5.0 (0.8)	4.8 (0.9)	5.2 (0.8)	5.0 (0.8)	0.36
Total	N (%)	192 (89)	24 (11)	47	263 (100)	

[a]ART=antiretroviral therapy; NNRTI=non-nucleoside reverse transcriptase inhibitor

With respect to the time-varying nature of adherence, our analytical approach is limited to only the first episode of treatment disruption, rather than multiple changes over time. Nevertheless, the time to first treatment disruption remains a clinically meaningful outcome, as

duration of time on initial therapy measures durability, tolerability, and general treatment response.

In addition, our preliminary analysis of changes in adherence over time does not indicate major changes in reported adherence over time. This constancy of response was both longitudinal across repeated questionnaires over time and similar across different questions. Comparing regimens across randomized arms by univariable logistic regression with generalized estimating equations for repeated measures over 192 weeks of follow-up, we found that point estimates leaned toward more difficult adherence to PI-based regimens than NNRTI-based regimens (Table 3.5). Thus, missed doses over the last 3 days likely represent characteristics of the participant-drug-adherence relationships over time and across questionnaire responses. Thus, the time to the first treatment disruption event is likely a meaningful outcome.

Table 3.5. Comparison of adherence questionnaire responses by ART regimen.

Adherence Measure	Odds Ratio (PI vs. NNRTI)[a]	95% Confidence Interval
Poor Adherence in Past 3 Days	1.34	0.81-2.23
Adherence Problems in Past 2 Weeks	1.21	0.68-2.17
Difficulty Remembering to Give Medications	1.18	0.59-2.36
Medications Interfere with Child's Everyday Life	1.45	0.88-2.40

[a]PI=protease inhibitor-based regimen; NNRTI=non-nucleoside reverse transcriptase inhibitor-based regimen

Finally, the heterogeneity of adherence questionnaires across networks remains a limitation. We will focus on the most harmonized portion of the questionnaire, missed doses over the prior 3 days. We will work to harmonize the remainder of the questionnaire on reasons for non-adherence over the prior 14 days, but such harmonization will likely be imperfect.

C8. Data Interpretation

We will interpret results based on hazard ratios relative to the null of 1 and the precision by width of 95% confidence intervals (95% CI). We will not rely on statistical significance. This study sample size was powered to evaluate this outcome using null-hypothesis testing.

The reasons for treatment disruption will be listed to help explain the primary results. Given the heterogeneity of questionnaire questions regarding reasons for treatment disruption, we will regard this analysis as only descriptive.

D. Aim 2: Estimate the Per-Protocol Effect of Initial PI versus NNRTI-Based ART on Time to Treatment Failure in Treatment-Naïve Children Living with HIV in Developed Countries in a Setting of Ideal Adherence.

D1. Study Design

We will perform a secondary analysis of data from the randomized PENPACT-1 trial estimating adherence-corrected differences in HIV RNA viral load control by initial PI- vs. NNRTI-based ART regimen. We will first replicate the trial's ITT analysis, substituting a primary outcome of viral failure over 4 years. Then, we will conduct a per-protocol analysis that accounts for protocol non-adherence by (1) censoring patients who cease their assigned therapy for non-medically indicated reasons and (2) correct for imparted selection bias using inverse-probability of censoring weights (IPCW), as described below.

D2. Intention-to-Treat versus Per-Protocol Estimate

The intention-to-treat (ITT) parameter estimates the effect of being assigned to a treatment protocol. In standard ITT analysis of clinical trials, poor adherence typically biases estimates of effective treatments towards the null, resulting in under-estimation of treatment efficacy and over-estimation of treatment safety.[102]

The per-protocol parameter estimates the effect of being assigned to, and remaining on, a treatment protocol. Robins and Finkelstein (RF) introduced inverse probability of censoring

weights (IPCW) to account for non-indicated treatment disruptions, whether by stoppage or switching of assigned therapy, or by study dropout; and describe a consistent estimator of the per-protocol effect.[95] This RF approach administratively censors study participants who cease to follow the assigned protocol, for instance, stopping or switching medications for reasons other than medical indications, such as adverse events. The approach then up-weights similar participants remaining under study to correct for induced informative censoring. The RF provides a principled way to address problematic post-randomization protocol violations, such as poor adherence and study dropout, which may affect clinical trial results.

D3. Treatment Assessment

The primary exposure will be the randomized initial PI- or NNRTI-based ART regimen, but we will define the population according to the original trial's ITT analysis, which excluded three (1%) of participants who were not started on ART. After performing the ITT analysis, we will perform a per-protocol analysis that corrects for post-randomization factors that define protocol deviations.

D4. Outcome Assessment

Our primary outcome will be time to treatment failure, defined as viral failure or clinical failure within 4 years of randomization. Time to viral failure will be defined at the first of two consecutive viral loads >400 copies/mL at or after Study Week 24 (≥20 weeks after randomization). Because the protocol only required viral load confirmation of viral failure at higher viral loads (>1,000 copies/mL or >30,000 copies/mL) and other factors leading to missed viral load measurements (such as dropout, not returning for labs, and protocol deviations), many viral loads >400 copies/mL were not confirmed within a short time frame. Thus, we will use the next measured viral load value, regardless of time interval, to confirm viral failure.

Clinical failure will be defined as clinical disease progression, namely a new Centers for

Disease Control and Prevention (CDC) stage C event (generally equivalent to a CDC stage 3 clinical event in the 2014 staging guidelines),[103,104] or other clinical progression such that the treating clinician believed that changing therapy was required prior to reaching the viral load switch point. Time to clinical failure will be defined at documentation of the event in the treatment record or case report form.

D5. Protocol Deviations

Protocol deviations will be defined as any non-medically indicated stoppages or changes of any component of the initially prescribed ART regimen that result in the participant receiving fewer than two NRTIs or not receiving the assigned class of PI or NNRTI at any time. The assigned class of PI or NNRTI on first line ART must be according to randomized arm; the assigned class on second line ART must be the reverse of the originally randomized class. Any switch to a third line or other regimen will be considered a protocol deviation. Participants receiving both a PI and an NNRTI at the same time or use of other classes, such as integrase inhibitors or fusion inhibitors, will be defined as protocol deviations, regardless of the reason for the prescription change.

ART medication stoppages or changes will be captured from both treatment records and adherence questionnaires. From treatment records, we will define medically indicated reasons for ART stoppages or changes as adverse events, viral failure, resistance, death, pregnancy, regimen simplification, or medication switches within the same antiretroviral class. During the parent study, nelfinavir underwent a drug recall. Any immediate switch to another drug within the same class for a nelfinavir recall or other drug supply problem will be defined as a non-protocol deviation, but any interruption of ART or switch to another drug class will be defined as a protocol deviation, as other drugs within the same class should have been available.

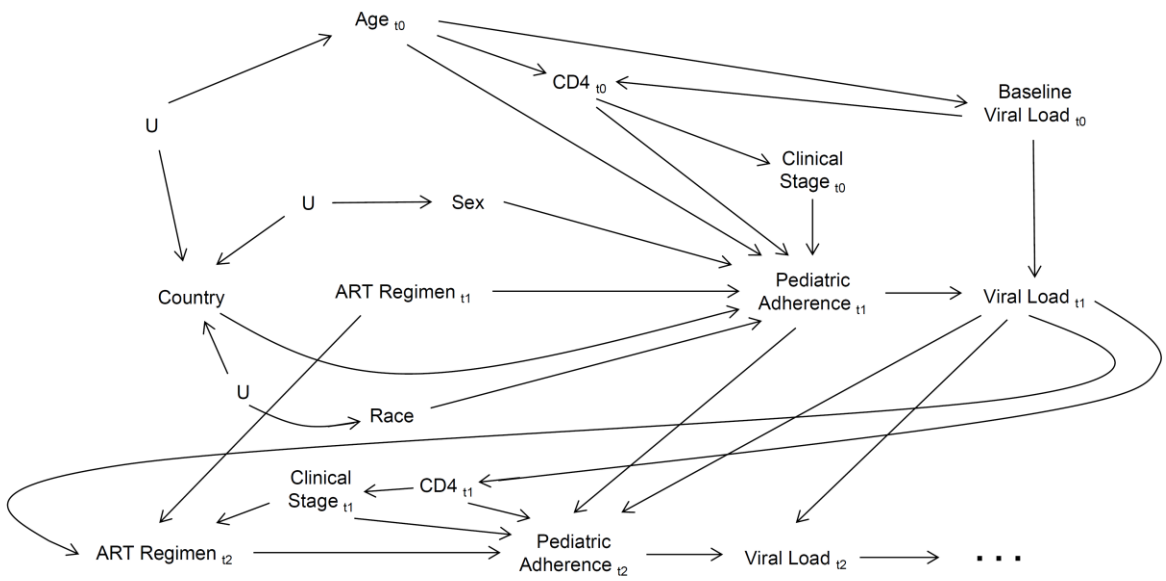
Because a medically indicated reason for ART stoppage or change would require a

clinician to determine the indication, any missed dose reported on the adherence questionnaire, regardless of reason, will be defined as a non-medically indicated stoppage. Study dropout, defined as cessation of viral load measurements before 4 years on study, will be counted as a protocol deviation. All non-medically indicated ART stoppages or changes and all dropouts will be defined as protocol deviations at their first occurrence.

D6. Covariate Assessment

To determine the most appropriate covariates, we constructed a causal diagram to analyze time-varying relationships among treatment regimen, protocol non-adherence, and viral load (Fig 3.2, Appendix IV Figure S2). The relevant paths in this analysis are those paths that affect both protocol non-adherence and viral load. Potential time-fixed covariates will include age at enrollment (<3 vs. 3-17 years), exposure to perinatal antiretrovirals, research network/region (PACTG/IMPAACT vs. PENTA), route of HIV exposure (vertical vs. other), baseline CDC clinical staging (stage C or not, categorized by the 1994 revised CDC classification system,[103] as used by the parent study), baseline CD4 cell count (stage 1 vs. 2 or 3, categorized according to 2014 CDC immunologic criteria[104]), and baseline $\log_{10}$ viral load. Potential time-varying covariates will include most recent categorized CD4 cell count from the prior visit, most recent $\log_{10}$ viral load from the prior visit, and an ART switch indicator variable. New HIV-related clinical manifestations on ART were not included because they were rare and may lead to positivity violations. Covariates ultimately included in models may be reduced based on covariate data, missingness, and strength of associations within the data.

Figure 3.2. Causal diagram relating ART regimen, adherence, viral load, and covariates.



All covariate data will be from the original data collected in the PENPACT-1 parent study. Variable coding will be defined according to CDC staging,[103,104] trial definitions, and exploration of data fit by splines[105] and polynomial expansion.

D7. Statistical Analysis

We will contrast PI vs. NNRTI treatment arms as randomized in the parent study's modified ITT analysis, which included only participants who started ART, and then perform a per-protocol analysis accounting for post-randomization protocol non-adherence in the same study population.

First, we will perform an unweighted analysis of the original data to generate modified ITT estimates. Follow-up will begin at the date of randomization. Participants will be right-censored at dropout or 4 years on study. Four years of follow-up will be defined as the week 204 visit plus a 6-week lag to capture late visits. Participants will be followed until their last documented viral load, viral failure, or 4 years, whichever occurred first.

Then to perform the per-protocol analysis, we will re-analyze the data using Robins-Finkelstein (RF) inverse probability-of-censoring weights (IPCW) applied to longitudinal studies with baseline randomization. Any participant who experiences a protocol deviation will be right-censored at that time. Then we will apply RF-IPCW to corrected for imparted informative censoring, as described by Robins and Finkelstein[95] and by Cain and Cole[88]:

Notation

Let

i index 1 to N = 263 participants

j index 1 to J = 210 weeks from ART initiation

R_{ij} = 1 indicate that participant i is at risk for treatment failure on day j,

0 otherwise

D_{ij} = 1 indicate that participant i experienced treatment failure at day j,

0 otherwise

C_{ij} = 1 indicate that participant i dropped out or became protocol non-adherent

before day j, 0 otherwise

X_i = 1 indicate that participant i was allocated to receive initial ART with a PI,

0 otherwise

Z_{ij} = 1 indicate that participant i was exposed to ART with a PI,

0 otherwise

$\bar{Z}_{ij}$ denote the history of Z_{ij} up through j, *i.e.* $\bar{Z}_{ij} = \{Z_{i0}, Z_{i1}, \ldots, Z_{ij}\}$

V_{ij} denote a vector of time fixed (*i.e.* at ART initiation, j = 0) and time-varying

covariates

$D_{ij}(\bar{z})$ = 1 indicate that participant i experienced viral failure on day j,

given participant i had exposure history $\bar{z}$, 0 otherwise

$D_{ij}(\bar{z})$ is a potential outcome, signifying an outcome which may be contrary to fact. We will assume exchangeability as no informative censoring given treatment allocation and measured time-fixed and time-varying covariates, formally defined as:

$$P(C_{ij} = 0 \mid X_i , \bar{V}_{ij-1}) = P[C_{ij} = 0 \mid X_i , \bar{V}_{ij-1} , D_{ij}(\bar{z})]$$

Robins-Finkelstein (RF) Inverse Probability-of-Censoring Weights

We will use RF inverse probability-of-censoring weights to correct for possible bias induced by dropout, death, or artificial censoring due to protocol non-adherence.

Weights W_{ij} are defined below:

If $C_{ij} = 1$ then $W_{ij} = 0$

If $C_{ij} = 0$ then $W_{ij} =$

$$\prod_{k=0}^{j} \frac{P(C_{ik} = 0 \mid X_i)}{P(C_{ik} = 0 \mid X_i , \bar{V}_{ik-1})}$$

Thus, on or after censoring, zero weights are applied. Prior to censoring, positive weights are applied. Positive weights will upweight uncensored patients (conditional on allocated ART and measured time-fixed and time-varying covariates) to compensate for imparted informative censoring, while stabilizing by the probability of remaining uncensored on day j conditional on allocated ART.

The conditional probabilities for the weights' numerators and denominators will be fit using pooled logistic regression models for the hazard of censoring as below:

logit $P(C_{ij} = 0 \mid X_i) = \alpha_{0j} + \alpha_1 X_i$ (weight numerator)

logit $P(C_{ij} = 0 \mid X_i , \bar{V}_{ij-1}) = \beta_{0j} + \beta_1 X_i + \beta_2' \bar{V}_{ij-1}$ (weight denominator)

where

α_{0j} and β_{0j} are week-specific intercepts

α_1 is the log hazard ratio for censoring comparing PI vs. NNRTI

β_1 is the log hazard ratio for censoring comparing PI vs. NNRTI among patients with the same covariate history $\overline{V}_{ij-1}$

β_2' is the transpose of the column vector of the log hazard ratio for the components of the covariate history matrix $\overline{V}_{ij-1}$

IPCW will be calculated separately for each treatment arm. Week-specific intercepts will be fit using splines fit according to the data by treatment arm to stabilize weights. If time-varying values are missing, we will carry forward the last observed value.

To clarify our approach, please note that the fitted pooled logistic regression will be used to estimate the probability of not being censored for protocol deviations using baseline and time-varying covariates for each participant at each week under study. This probability will be applied as the denominator of the IPCW, and week on randomization will be applied as the numerator to stabilize the weights.

By applying these weights, participants remaining under study will be up-weighted, using the inverse of the probability of remaining free from a protocol deviation, to replace an equivalent number of participants who were censored. Censoring and up-weighting will be applied cumulatively at the week of each protocol deviation until the end of 4 years on study. Similar to the modified ITT analysis, follow-up will begin at randomization, and participants will be right-censored without up-weighting at 4 years on study.

Regression Models

For the primary outcome, we will estimate the risk of viral failure using the complement of the Kaplan-Meier estimator, unweighted for modified ITT analysis and applying IPCW for the

per-protocol analysis. We will estimate the hazard ratio (HR) for viral failure using a Cox proportional hazards model, unweighted and weighted. Proportional hazards assumptions will be assessed graphically, using time interaction terms, and with martingale residuals. In adjusted analyses, the ITT analysis will be stratified by baseline randomized stratification variables: age, exposure to perinatal antiretrovirals, and research network (which varied by region). For the per-protocol analysis conditional on randomized stratification variables, we will include the randomized stratification variables to the final reduced model for IPCW. All confidence intervals will be estimated using the robust sandwich variance estimator with an independent working covariance matrix.

Finally, we will assess the sensitivity of our results to changes in our model specifications. Given the relation between time-varying viral load from the prior visit and viral failure, an alternative IPCW model will excluded time-varying viral load. Other alternative IPCW models will exclude the weakest predictor(s) of censoring and include only randomized stratification variables. Sensitivity analyses will assess the extremes of corrections for censoring protocol deviations by (1) estimating effects without applying IPCW; (2) assuming all censored participants in the PI arm are failures and all censored participants in the NNRTI arm are non-failures; and (3) reversing this latter coding.

Analyses will be conducted in SAS® version 9.4 (Cary, NC).

D8. Potential Limitations

Although PENPACT-1 is one of the largest pediatric HIV trials, the sample size may still limit the number of covariates that may be included in the model, potentially presenting tensions between sufficient covariate control for exchangeability versus maintaining positivity. Moreover, the study will suffer from measurement error from incomplete capture of treatment disruptions on the treatment record or inaccurate reporting of non-adherence on the questionnaire. Finally,

the traditional viral failure outcome has limitations as (1) a composite outcome that combines distinct events of viral suppression and viral rebound and (2) assignment of pre-determined failure time, e.g. 24 weeks of ART, for patients who fail to achieve viral suppression, rather than a more specific time to suppression.

D9. Addressing Potential Limitations

Regarding he limited sample size potentially compromising exchangeability and positivity assumptions, this limitation cannot be corrected. We accept this limitation but expect that we will have improved validity of this method over methods that would not account for post-randomization protocol deviations.

As to the measurement error resulting from treatment records and adherence questionnaires, the composite variable of protocol non-adherence from either measure should decrease the measurement error from either instrument alone. In addition, the validity of the questionnaire has previously been evaluated,[61] and our preliminary data suggests some predictive value of the questionnaire (See Section C7). Measurement error is inherent in any adherence measure, and we are left with the adherence measure used in the study.

Finally, if the data suggest a need to separate the composite outcome of viral failure, then we will employ the method proposed by Gouskova *et. al.*, which uses an endpoint based on the probability of being virally suppressed.[106] This method estimates the difference in survival functions for viral suppression and viral rebound and may be used to estimate differences in mean total time of viral suppression. We will use this method if the proposed traditional composite endpoint suggests an interaction across time, such as survival functions crossing one another. An example of this scenario would be if one treatment arm has a lower probability of viral suppression initially, then has a higher probability of viral rebound later in the follow-up period.

D10. Data Interpretation

Our data interpretation will focus on the shift in hazard ratios and 95% CIs from the ITT to the per-protocol analysis. The ITT analysis would estimate treatment efficacy in the setting where protocol non-adherence is permitted. The per-protocol analysis estimates treatment efficacy in the setting of participants adhering to the prescribed protocol. In this case, shifts in ITT to per-protocol estimates would indicate how robust the PI or NNRTI ART regimens are to non-adherence. Greater shifts would suggest that non-adherence has a greater influence on treatment failure. We will interpret results under assumptions of ITT, per-protocol, and the degree of shift between the two analyses.

E. Conclusion

Adherence to ART is a major determinant of pediatric HIV treatment outcomes. Although many approaches to improving adherence have been proposed, prescribing patterns are the most modifiable by clinicians. PIs and NNRTIs are key drugs that may be modified, but the relationships are not well-defined among drug, adherence, and viral control. Therefore, we propose to disentangle drug-adherence and adherence-viral control relationships in a randomized controlled trial of PI vs. NNRTI ART in children. The results generated may help determine the influence of prescription decisions for HIV-infected children on time to treatment disruption and time to treatment failure when accounting for these treatment disruptions.

CHAPTER 4: TIME TO TREATMENT DISRPTION IN CHILDREN RANDOMIZED TO PROTEASE INHIBITOR VERSUS NON-NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITOR REGIMENS

A. Overview

Background: Choice of initial antiretroviral therapy (ART) regimen may help HIV-infected children maintain optimal, continuous therapy. We assessed treatment-naïve children for differences in time to treatment disruption across randomly-assigned protease inhibitor (PI) versus non-nucleoside reverse transcriptase inhibitor (NNRTI)-based initial ART.

Setting: We performed a secondary analysis of a multicenter phase 2/3, randomized, open-label trial in Europe, North and South America from 2002-2009.

Methods: HIV-1-infected, ART-naïve children aged 31 days to <18 years were randomized to ART with two nucleoside reverse transcriptase inhibitors plus a PI or NNRTI. Time to first documented treatment disruption to any component of ART, derived from treatment records and adherence questionnaires, was analyzed using Kaplan-Meier estimators and Cox proportional hazards models.

Results: The modified intention-to-treat analysis included 263 participants. Seventy-two percent (n = 190) of participants experienced ≥1 treatment disruption during study. At 4 years, treatment disruption probabilities were 70% (PI) vs. 63% (NNRTI). The unadjusted hazard ratio (HR) for treatment disruptions comparing PI vs. NNRTI-based regimens was 1.19, 95% confidence interval [CI] 0.88-1.61 (adjusted HR 1.24, 95% CI 0.91-1.68). By study end, treatment disruption probabilities converged (PI 81%, NNRTI 84%) with unadjusted HR 1.11, 95% CI 0.84-1.48 (adjusted HR 1.13, 95% CI 0.84-1.50). Reported reasons for treatment

disruptions suggested that participants on PIs experienced greater tolerability problems.

Conclusions: Children had similar time to treatment disruption for initial PI- and NNRTI-based ART, despite greater reported tolerability problems with PI regimens. Initial pediatric ART with either a PI or NNRTI may be acceptable for maintaining continuous therapy.

B. Introduction

Globally, 1.8 million children are living with HIV, and 110,000 die annually due to AIDS-related illnesses.[1] For HIV-infected children, greatest survival outcomes can be achieved only with optimal, uninterrupted treatment on effective antiretroviral therapy (ART). Treatment disruptions, defined as any interruption or alteration of initial ART, may result from patient-level factors (*e.g.*, poor adherence, drug intolerance), provider-level factors (*e.g.*, prescription stops, changes, or errors), or systems-level factors (*e.g.*, stock outs, interruptions in drug delivery). Unfortunately, treatment disruptions may result in treatment failure, acquisition of resistance mutations, and loss of future treatment options—which are particularly consequential in children. Compared with adults, children have greater pharmacokinetic variability and fewer available licensed drugs.[4,85] Due to longer lifetime antiretroviral exposure, children have more potential for long-term toxicity.[16,17] Children have greater social vulnerability related to their dependence on others for medical care and medication administration.[20,107] If inadequately treated, children progress more rapidly to HIV disease and death.[2,86,108] As children's initial ART regimens are often their best opportunity for effective, tolerable treatment, longer time on their initial regimen generally means greater efficacy, fewer toxicities, and more lifetime treatment options. Analyzing longitudinal relationships between pediatric ART regimens and time to treatment disruption allows identification of initial ART regimens that pose greater challenges to maintaining optimal, continuous ART.

When deciding which regimen to prescribe to optimize clinical outcomes, clinicians must

consider both drug pharmacology and potential adherence to ART regimens.[109] Boosted protease-inhibitor (PI)-based regimens appear more forgiving of treatment disruptions than do non-nucleoside reverse transcriptase inhibitor (NNRTI)-based regimens.[55,57,60,96,98] However, certain PI characteristics decrease adherence and tolerability, particularly in children: poor taste; gastrointestinal toxicity; and regimen complexity, such as pill burden, storage requirements, and dosing frequency.[18-20,41,60,110,111] Prior pediatric studies that have assessed the ability of children to maintain continuous therapy did not do so in settings in which use of PI- vs. NNRTI-based ART regimens was randomly allocated, nor have prior studies measured treatment disruptions longitudinally. As a result, these previously conducted studies have potential for residual confounding from unmeasured covariates. Furthermore, most studies have isolated analyses of prescription patterns, adherence, and tolerability, rather than evaluating the total effect of the regimen on maintaining optimal, continuous therapy. In the PENPACT-1 study, 266 HIV-1-infected, treatment-naïve children from Europe, North America, and South America were randomized to ART with either a PI or NNRTI and followed longitudinally for at least 4 years.[45] We aimed to assess PENPACT-1 participants for differences in time to treatment disruption across randomized PI vs. NNRTI treatment arms at 4 years and end of study.

C. Methods

C1. Study Design and Participants

PENPACT-1 (PACTG 390 / PENTA 9) was an international multicenter phase 2/3, randomized, open-label trial enrolling children infected with HIV-1 from 68 clinical centers in 13 countries in Europe and North and South America between September 25, 2002, and September 7, 2005.[45] Eligible children aged 31 days to less than 18 years were HIV-1-infected and had not received ART or received only antiretrovirals for <56 days to reduce mother-to-child transmission (excluding single-dose nevirapine). All parents or guardians and children, as

appropriate, gave written consent for the parent trial; this protocol was conducted in accordance with the Declaration of Helsinki and approved by the relevant ethics committee or institutional review board (IRB) for each participating center. The secondary analysis on time to treatment disruption was deemed exempt by the Duke University IRB and approved by the University of North Carolina-Chapel Hill and Children's Mercy Kansas City IRBs. This study is registered with the International Standard Randomised Controlled Trial Number Registry (ISRCTN73318385).

Children were randomized 1:1 to start ART with two nucleoside reverse transcriptase inhibitors (NRTIs) plus either a PI or NNRTI. The study was open label, and the treating clinician chose the two NRTI drugs combined with a drug from the randomly assigned PI or NNRTI class. Children underwent clinical and HIV-1 RNA viral load assessments at randomization (week 0), weeks 2, 4, 8, 12, 16, 24, and then every 12 weeks until the last child assigned to treatment reached 4 years of follow-up (August 31, 2009). Treatment starts, changes, and stoppages were recorded at clinical visits and *ad hoc* throughout the study. Trained study personnel administered validated adherence questionnaires every 24 weeks after randomization.[61] Adherence questionnaires recorded the number of missed doses to all antiretrovirals over the 3 days prior to visit and barriers to adherence experienced within 2 weeks prior to visit. Four years of follow-up was defined as the week 192 visit plus a 6-week lag to capture late visits.

C2. Outcomes

We defined time to treatment disruption as the number of weeks between randomization and the first documented treatment disruption event. We defined treatment disruption as stopping, switching, or reporting missed doses of any component of the initial ART regimen for any reason except recall of nelfinavir (June 2007) or planned treatment interruptions.

Information on ART stoppages or switches was derived from participants' treatment records, and missed doses were defined as any questionnaire-reported missed doses within 3 days prior to the study visit.

Additional analyses included adjustment for stratified randomization factors (age, receipt of perinatal ART prophylaxis, research network), assessed differences in outcome for the primary follow-up time point (4 years) vs. the entire study, and explored reasons for treatment disruptions. Reasons for treatment disruptions were analyzed using (1) the treatment record's documented rationale for ART stop or change and (2) any questionnaire-reported barriers to adherence within 2 weeks prior to the visit when missed dose(s) were reported.

We assessed the sensitivity of our results to missing questionnaires by evaluating alternative definitions of treatment disruption. Our alternative outcome definitions included: including two consecutive missing adherence questionnaires (plus a 6-week lag for a late visit) as a treatment disruption, and right-censoring after two consecutive missing adherence questionnaires.

C3. Statistical Analysis

PI vs. NNRTI treatment groups were assessed according to a modified intention-to-treat (ITT) analysis consistent with the original study.[45] The sole modification was removal of three participants: two who withdrew consent prior to ART initiation, and one with a major eligibility violation. Follow-up began at date of randomization. Participants were right-censored for initial treatment contrary to randomization, planned treatment interruption, death, withdrawal of consent, loss to follow-up, or study end.

For the primary outcome, we estimated the risk of treatment disruptions using the complement of the Kaplan-Meier estimator. We estimated the hazard ratio for treatment disruptions using Cox proportional hazards models. Proportional hazards assumptions were

assessed graphically, using time-interaction terms, and with martingale residuals. In adjusted analyses, we stratified by baseline randomized stratification variables: age, exposure to perinatal ART, and research network (which varied by region). Analyses were conducted in SAS® version 9.4 (Cary, NC).

D. Results

PENPACT-1 enrolled 266 HIV-1 infected children from 68 centers in 13 countries in Europe, North America, and South America. The modified ITT analysis was restricted to 263 participants who initiated ART. Participants were a median age of 6.5 years at enrollment (IQR [interquartile range], 1.8-12.9), 52% male, 49% black, and 79% exposed to HIV via vertical transmission (Table 4.1). Fifty-one percent had moderate to severe clinical symptoms (CDC stage B or C). Median growth parameters were below average (weight-for-age Z score -0.6; height-for-age Z score -0.9). Median CD4 Z-score was -3.5, consistent with predominance of moderate to severe immunosuppression, and median viral load was 5.0 $\log_{10}$ copies/mL. Whereas 15% of children had ART exposure for prevention of mother-to-child transmission, 4% had at least one major resistance mutation at baseline. Although treatment groups had differences in racial distribution, baseline characteristics relating to mode of HIV-1 acquisition, clinical and immunological status, and ART resistance were generally balanced across ART regimens, consistent with the randomized design.

Median follow-up time was 261 weeks (IQR, 217-313). Two participants in each arm were started on a PI or NNRTI contrary to randomization; two underwent planned treatment interruption; five withdrew from study after ART initiation; 37 were lost to follow-up; and one patient died, due to HIV-related complications (Figure 5.1). Two hundred forty-nine participants ever completed an adherence questionnaire, totaling 2,112 questionnaires over the duration of the study for a mean of 8.5 questionnaires per participant.

Overall, 191 of 263 participants had at least one treatment disruption event during the study, with 66% treatment disruption probability at 4 years (primary follow-up period) and 83% treatment disruption probability at study end (6.5 years). At 4 years, probabilities of treatment disruption were 70% vs. 63% in the PI and NNRTI arms, respectively (Figure 5.2). Hazards for treatment disruption, however, were similar for PI vs. NNRTI-based regimens (unadjusted hazard ratio [HR] 1.19, 95% confidence interval [CI] 0.88-1.61), even after adjustment for stratification factors of age, receipt of perinatal ART, and research network/region (adjusted HR 1.24, 95% CI 0.91-1.68) [Table 4.2].

After 4 years, treatment disruption probabilities converged, such that treatment disruption probabilities at study end were 81% for PI vs. 84% for NNRTI arms, but changes over time in the hazard ratio of treatment disruption by treatment arms were non-significant (unadjusted P for interaction = 0.33, adjusted P = 0.21). Hazards for treatment disruption over the entire study period were similar for PI vs. NNRTI-based regimens, unadjusted (HR 1.11, 95% CI 0.84-1.48) and adjusted (HR 1.13, 95% CI 0.84-1.50).

Of 191 treatment disruption events, 126 events were based on ART regimen stoppages or changes in the treatment record, and 67 events were reported missing doses on adherence questionnaires, with two participants experiencing both event types simultaneously. Of the treatment stops or changes, 25% of events were substitutions of at least one first-line ART drug (PI 32%, NNRTI 16%), 53% were stoppage or suspension of the entire first-line ART regimen (PI 48%, NNRTI 59%), and 22% were switches to a second-line ART regimen (PI 20%, NNRTI 25%). Most frequent reasons documented for ART stops or changes were adverse events (34%), viral failure (22%), caregiver request (18%), non-adherence (7%), and temporary break (6%), with the greatest difference between PIs over NNRTIs for adverse events (Table 4.3).

Reports of missed doses on adherence questionnaires were balanced between PI and NNRTI arms, as 35% of non-adherence events in each arm were from patient or caregiver reports. The most common questionnaire-reported barriers to adherence, forgetting/lacking support (30%) or running out of medications (25%), were balanced between PI and NNRTI regimens. Other common questionnaire-reported adherence problems—including difficulties with administration, such as those attributed to intolerance, taste, patient refusal (24%); fear of disclosure to others (22%); patient refusal (21%); difficulties with scheduling or lifestyle (18%); and concerns about drug toxicity (16%)—were more frequently reported in participants in the PI arm (Table 4.3).

Accounting for missing questionnaires by including at least two consecutive missed questionnaires as a treatment disruption event yielded 4-year hazard ratios close to the null (unadjusted HR 1.06, 95% CI 0.80-1.40; adjusted HR 1.08, 95% CI 0.81-1.43). Right-censoring participants with two consecutive missing adherence questionnaires produced 4-year hazard ratios similar to the primary analysis (unadjusted HR 1.20, 95% CI 0.88-1.63; adjusted HR 1.26, 95% CI 0.92-1.72).

E. Discussion

In PENPACT-1, time to treatment disruption was similar in patients randomized to PIs and NNRTIs. Point estimates were mildly in the direction of more treatment disruptions in PI-based regimens, particularly in the primary end point of 4 years, but differences were small, possibly due to chance, and appeared to decrease by study end. Exploration of reasons for treatment disruptions suggested that PI-based regimens may be less tolerable, both due to adverse events leading to treatment stoppages or substitutions and to regimen-specific adherence barriers reported on the adherence questionnaire. However, these PI-associated difficulties did not interrupt continuous therapy to the initial PI-based regimens more than they did to NNRTI-

based regimens.

Although we did not find a meaningful difference in treatment disruptions in PI vs. NNRTI-based regimens, the secondary analyses exploring reasons for treatment disruptions suggested that administration of a PI-based regimen to a child may be a struggle, even if not resulting in actual missed doses. The treatment record suggested that participants experienced more adverse events to PIs over NNRTIs, but adherence questionnaire responses formed a pattern of difficulties with PI tolerability, whether attributed to taste, medication volume or pill burden, toxicity, or simply patient refusal. This pattern would be consistent with existing literature on PI vs. NNRTI regimens. PIs have higher drug toxicity, especially gastrointestinal side effects, and intolerance, particularly regarding their noxious taste.[18-20,41-44] Even if children are able to swallow pills, certain PIs are available only as large pills.[89,112] No PIs are available as complete-regimen combinations for children, whereas single-tablet NNRTI regimens can facilitate adherence through administration of fewer pills.[4,52,90-93] Participants reported more barriers to adherence in PIs related to scheduling or lifestyle interference, which may relate to dosing frequency. We hypothesize that increased fear of disclosure to others, as noted in the PI arm, may relate to difficulties concealing drug administration when given more frequently. Higher dosing frequency has been associated with more frequent treatment disruptions.[18,48-52,90,92] Some NNRTIs, most notably efavirenz, have more suitable pharmacokinetics for once daily administration. In our study, most PI-based regimens were administered at least twice daily, whereas some commonly used NNRTI-based regimens allowed once-daily dosing.

Most children in PENPACT-1 experienced a treatment disruption event during the study. Only about one-third of participants remained continuously on their initial ART at 4 years; only one-sixth remained continuously on initial ART at study end. Maintaining continuous therapy on

ART is critical to sustained HIV-related outcomes, as suppressing viral load decreases the probability of HIV sub-populations acquiring antiretroviral resistance mutations and chances of forward infection.[5,22,23,32-35,113,114] Although optimal adherence targets vary by PI vs. NNRTI class, adherence has been modest across ART studies, especially patients failing to achieve viral suppression.[36,37,39,40,52-56,60,109] Notably, ART appears to be less successful in producing viral suppression in children, who are more prone to viral failure and resistance due to higher plasma viral loads, less robust antiviral immune responses, greater pharmacokinetic variability, and social dependency.[5,21] Adolescents have particularly worse viral and immunological outcomes, due to poor ART adherence.[22-25,115] The large proportion of children in PENPACT-1 with disruptions of their initial ART raises concerns regarding long-term durability, especially as these patients were receiving adherence support on a clinical trial protocol at specialty pediatric HIV centers.

Based on our data, choice of an initial PI- vs. NNRTI-based regimen may not have a major impact on ART treatment disruption. Despite differences in reported regimen-related adherence barriers, participants in both treatment arms persevered in taking their regimens similarly. Moreover, the most common questionnaire-reported barriers were not regimen-specific: forgetting/lack of support and running out of drug. Novel interventions may still be able to improve the experience of drug administration. Pediatric granules using nanotechnology may improve palatability and decrease pill burden, and precision medicine related to taste-sensing genotypes may hold promise for prescribing according to individualized palatability.[116,117] In adult data, integrase strand transferase inhibitors (INSTIs) have been at least as tolerable as PIs or NNRTIs, if not more so, and INSTIs are increasingly preferred drugs in children.[101,118-120] Nevertheless, a primary goal of optimizing continuous therapy to ART is durable viral

suppression, which was comparable across PI vs. NNRTI arms in this study's parent trial, although similar trials had variable results.[45,84,99,100,121,122] In this study population, choice of either PI- or NNRTI-based initial ART appears acceptable.

Our estimates of treatment disruption may have had measurement error. First, we had no direct measures of drug exposure, such as therapeutic drug monitoring. Treatment records captured only prescribing events and documented ART disruptions, and the adherence questionnaires relied on accurate reporting by either the child or the caregiver, if present and willing to answer. Although we relied on a questionnaire that has previously been validated,[61] reporting biases and unanswered questionnaires may have affected our measures of missed doses. Our combining treatment records and adherence questionnaires into a composite outcome should have decreased measurement error from either instrument individually. Second, adherence questionnaires in this study focused on ART adherence over the 3 days prior to the most recent visit and inquired about adherence barriers encountered over the prior 2 weeks, rather than a daily measure of adherence throughout the study. The time-varying nature of treatment disruption[47,123] means that patients may have experienced an initial or temporary period of treatment disruption that was subsequently corrected, but our analysis presents only data on time to first event of treatment disruption. Third, limited participant report of individual drugs missed on the adherence questionnaire precluded definitive identification of treatment disruptions of individual drugs. Instead, we assessed treatment disruption to any component of the ART regimen. Fourth, heterogeneity of adherence questionnaires across networks, ages, and respondents regarding barriers to therapy should caution against rigorous interpretation of reasons for treatment disruptions. Finally, this study size was not sufficient to distinguish differences on the order of 7%, as was seen at 4 years.

F. Conclusion

In conclusion, children in PENPACT-1 had similar time to treatment disruption for initial PI-based regimens and NNRTI-based regimens. Although secondary analyses suggest that PI-based regimens may be more difficult to tolerate and may be less convenient to administer, these difficulties did not result in a large difference in children stopping, changing, or missing doses at 4 years (PI 70%, NNRTI 63%), and any suggested differences diminished by study end (PI 81%, NNRTI 84%). Initial ART with either a PI or NNRTI may be acceptable for maintaining continuous therapy on ART in children.

Figure 4.1. Study profile.

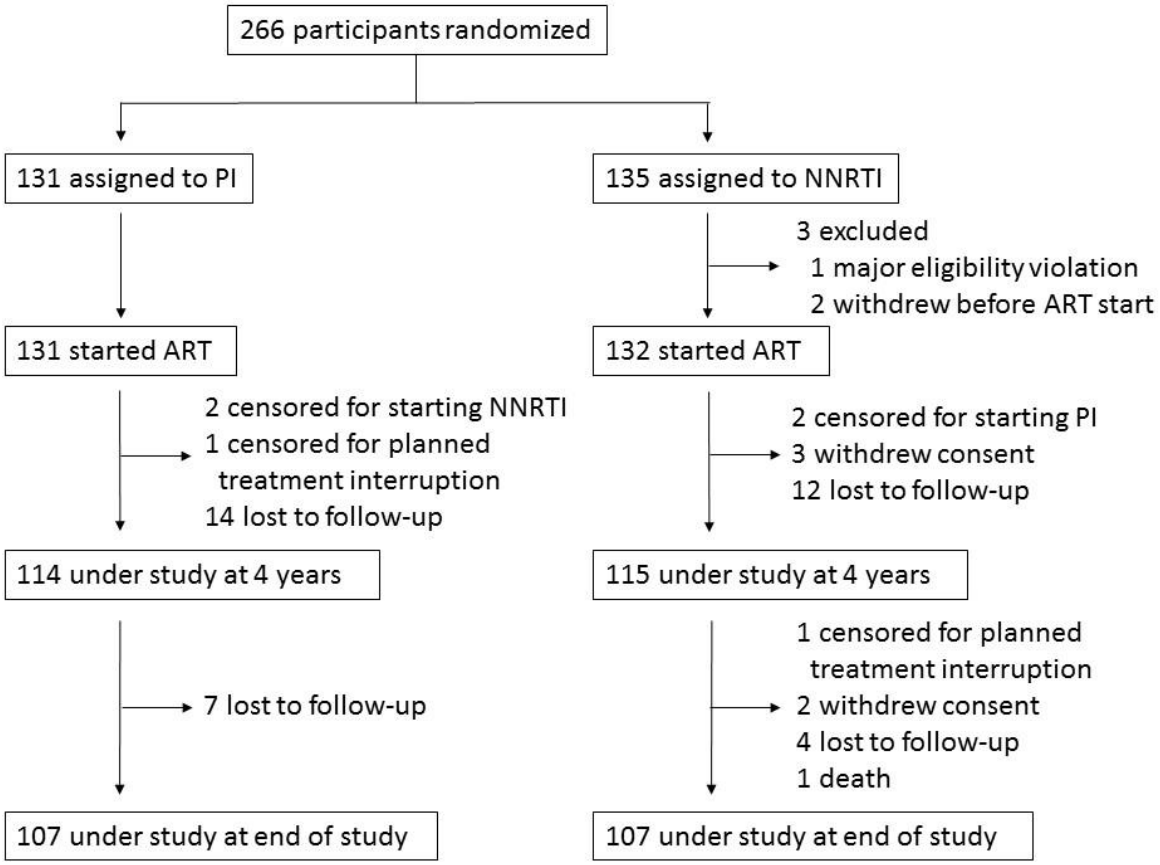


Figure 4.2. Proportion of children experiencing treatment disruption from initial ART regimen by study week. The vertical line delineates 4 years on study.

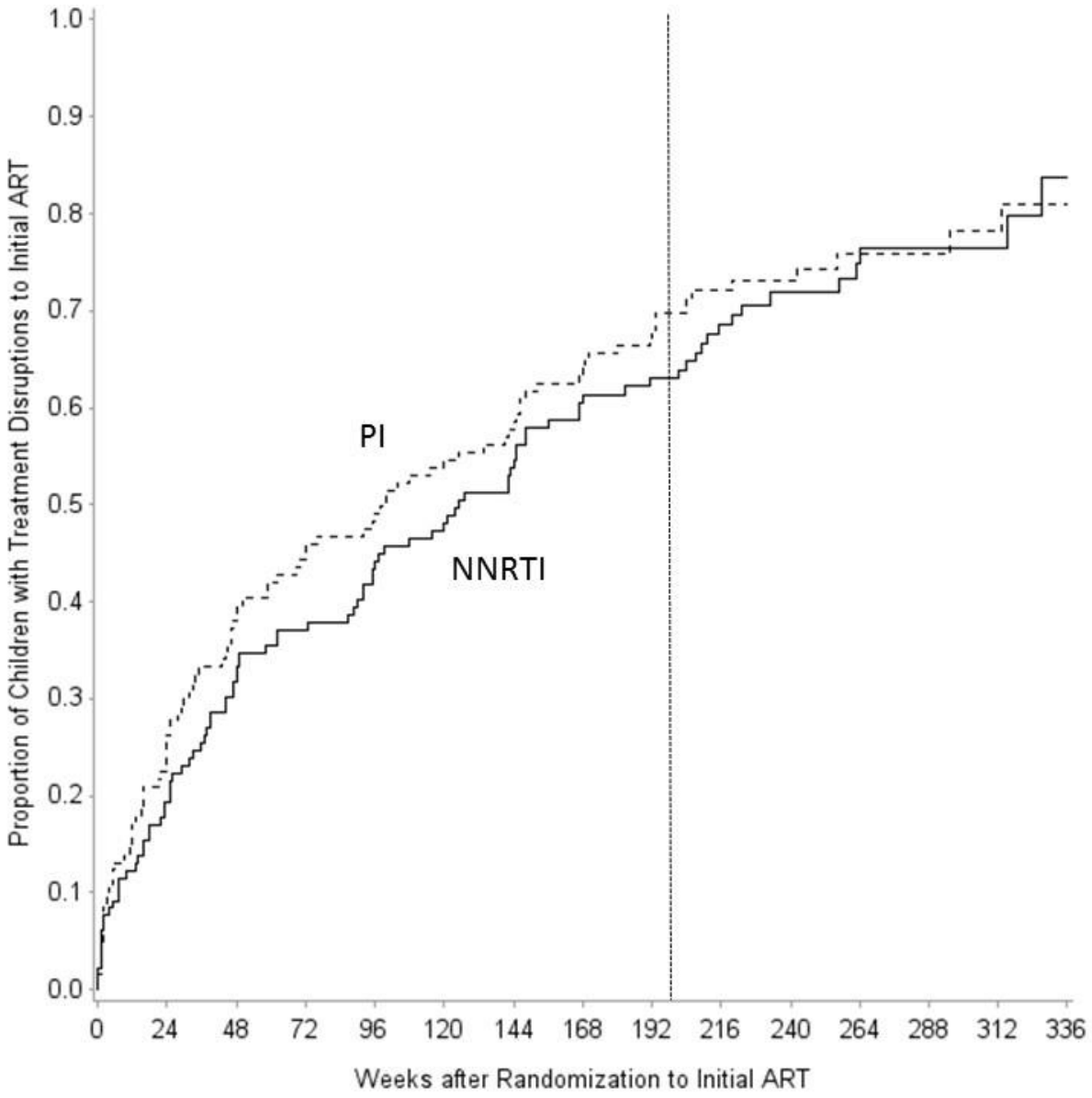


Table 4.1. Baseline characteristics of study participants according to initial ART regimen.

Variable		Randomized Group		
		PI	NNRTI	Total
N		131	132	263
Age				
<3 years	*n* (%)	34 (26%)	36 (27%)	70 (27%)
3-17 years	*n* (%)	97 (74%)	96 (73%)	193 (73%)
Age in years	Median (IQR)	7.1 (2.8, 13.7)	6.4 (2.7, 11.0)	6.5 (2.8, 12.9)
Sex				
Male	*n* (%)	69 (53%)	67 (51%)	136 (52%)
Race				
Black, Non-Hispanic	*n* (%)	60 (46%)	69 (52%)	129 (49%)
White, Non-Hispanic	*n* (%)	40 (31%)	29 (22%)	69 (26%)
Hispanic/Other	*n* (%)	31 (24%)	34 (26%)	65 (25%)
Research Network[a]				
PENTA	*n* (%)	95 (73%)	93 (70%)	188 (71%)
PACTG/IMPAACT	*n* (%)	36 (27%)	39 (30%)	75 (29%)
Route of Infection				
Vertical	*n* (%)	103 (79%)	106 (80%)	209 (79%)
Other/Unknown	*n* (%)	28 (21%)	26 (20%)	54 (21%)
CDC Clinical Stage				
N	*n* (%)	27 (21%)	29 (22%)	56 (21%)
A	*n* (%)	35 (27%)	37 (28%)	72 (27%)
B	*n* (%)	41 (31%)	43 (33%)	84 (32%)
C	*n* (%)	28 (21%)	23 (17%)	51 (19%)
Weight-for-Age Z-score	Median (IQR)	-0.5 (-1.6, 0.1)	-0.7 (-1.6, 0.2)	-0.6 (-1.6, 0.1)
Height-for-Age Z-score	Median (IQR)	-0.9 (-1.5, -0.2)	-0.9 (-1.8, 0)	-0.9 (-1.7, -0.2)
CD4 Z score	Median (IQR)	-3.6 (-7.2, -1.7)	-3.4 (-6.5, -1.4)	-3.5 (-6.8, -1.6)
Viral Load $\log_{10}$ copies/mL	Median (IQR)	5.1 (4.5, 5.7)	5.0 (4.5, 5.6)	5.0 (4.5, 5.7)
Perinatal ART Exposure	*n* (%)	19 (15%)	20 (15%)	39 (15%)
≥1 Major Resistance Mutation[b]	*n/N* (%)	5/116 (4%)	5/123 (4%)	10/239 (4%)
HIV-1 subtype				
B	*n* (%)	52 (42%)	49 (39%)	101 (41%)
C	*n* (%)	13 (11%)	12 (10%)	25 (10%)
F	*n* (%)	25 (20%)	23 (18%)	48 (19%)
A/CRF_AG/D/G	*n* (%)	21 (17%)	31 (25%)	52 (21%)
Unclassified	*n* (%)	12 (10%)	11 (9%)	23 (9%)
Switching Threshold				
1,000 copies/mL	*n* (%)	66 (50%)	68 (52%)	134 (51%)
30,000 copies/mL	*n* (%)	65 (50%)	64 (48%)	129 (49%)
Duration of Follow-Up in weeks	Median (IQR)	263 (217, 313)	260 (219, 316)	261 (217, 313)

Abbreviations: ART, antiretroviral therapy; IQR, interquartile range; *N*, total sample size; *n*, subsample size; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor.

[a] PENTA sites were predominantly in Europe, South America, and the Bahamas. PACTG/IMPAACT sites were based primarily in the United States.

[b] Not all patients had successful baseline genotypic resistance assays.

Table 4.2. Hazard ratios of treatment disruption comparing initial PI- vs. NNRTI-based regimens.

Outcome Measure	Unadjusted Hazard Ratio (95% CI)	Adjusted Hazard Ratio (95% CI)
Treatment disruption within 4 years	1.19 (0.88-1.61)	1.24 (0.91-1.68)
Treatment disruption by end of study (6.5 years)	1.11 (0.84-1.48)	1.13 (0.84-1.50)

Abbreviations: NNRTI=non-nucleoside reverse transcriptase inhibitor-based regimen; PI=protease inhibitor-based regimen.

Table 4.3. Reasons listed for treatment disruption events.

Reason / Barrier		PI	NNRTI	Total
Treatment Record[a]				
Adverse event	*n* (%)	24 (37%)	19 (31%)	43 (34%)
Viral failure	*n* (%)	13 (20%)	15 (25%)	28 (22%)
Caregiver request	*n* (%)	11 (17%)	12 (20%)	23 (18%)
Non-adherence	*n* (%)	6 (9%)	3 (5%)	9 (7%)
Temporary break	*n* (%)	3 (5%)	5 (8%)	8 (6%)
Unknown	*n* (%)	5 (8%)	1 (2%)	6 (5%)
Drug supply problem	*n* (%)	1 (2%)	2 (3%)	3 (2%)
Intercurrent illness	*n* (%)	0 (0%)	2 (3%)	2 (2%)
Resistance	*n* (%)	1 (2%)	1 (2%)	2 (2%)
Parent forgot	*n* (%)	1 (2%)	0 (0%)	1 (1%)
Simplification	*n* (%)	0 (0%)	1 (2%)	1 (1%)
Treatment record total	*n*	65	61	126
Adherence Questionnaire[b]				
Forgot/lack of support	*n* (%)	10 (29%)	10 (30%)	20 (30%)
Ran out of drug	*n* (%)	8 (24%)	9 (27%)	17 (25%)
Problems taking some of the drugs (*e.g.*, intolerance, taste, medication volume)	*n* (%)	11 (32%)	5 (15%)	16 (24%)
Fear of disclosure to others	*n* (%)	10 (29%)	5 (15%)	15 (22%)
Patient refused/didn't want to take drugs	*n* (%)	10 (29%)	4 (12%)	14 (21%)
Scheduling/lifestyle interference	*n* (%)	9 (26%)	3 (9%)	12 (18%)
Drug toxicity concerns	*n* (%)	7 (21%)	4 (12%)	11 (16%)
Supervised by someone else or multiple caregivers	*n* (%)	6 (18%)	5 (15%)	11 (16%)
Patient unwell	*n* (%)	6 (18%)	4 (12%)	10 (15%)
Other	*n* (%)	4 (12%)	5 (15%)	9 (13%)
Different routine/change in living situation	*n* (%)	3 (9%)	4 (12%)	7 (10%)
Fed up giving/taking drugs	*n* (%)	3 (9%)	2 (6%)	5 (7%)
Think medication is not needed or not helping	*n* (%)	2 (6%)	2 (6%)	4 (6%)
Caregiver unwell/depressed	*n* (%)	0 (0%)	0 (0%)	0 (0%)
Total listed problems on questionnaire[b]	*n*	89	62	151
Total participants with questionnaire-reported missed doses	*n*	34	33	67
Total Treatment Disruption Events[c]	*n*	97	94	191

Abbreviations: *n*, subsample size or number of events; NNRTI=non-nucleoside reverse transcriptase inhibitor; PI=protease inhibitor.

[a] One category allowed per treatment record change or stop.

[b] Participants may have answered in more than one category.

[c] Some participants had both a treatment record and adherence questionnaire event at the same time.

CHAPTER 5: PER-PROTOCOL EFFECT OF PROTEASE INHIBITOR VERSUS NON-NUCLEOSIDE REVEERSE TRANSCRIPTASE INHIBITOR REGIMENS IN CHILDREN LIVING WITH HIV

A. Overview

Adherence to antiretroviral therapy predicts outcomes of pediatric antiretroviral therapy. We aimed to estimate the per-protocol effect of initial protease inhibitor versus non-nucleoside reverse transcriptase inhibitor-based antiretroviral therapy in treatment-naïve children living with HIV in developed countries in a setting of ideal adherence. We used data from the PENPACT-1 trial, which randomized children to initial therapy with either of these regimens starting in 2002 and followed until the last enrollee reached 4 years of follow-up in 2009. We performed an intention-to-treat analysis to estimate time to treatment failure. Then we generated per-protocol estimates by administratively right-censoring participants who experienced non-medically indicated treatment disruption or dropout and used inverse-probability of censoring weights to correct for imparted informative censoring. In the intention-to-treat analysis, participants on protease inhibitors experienced a 4-year probability of treatment failure of 41.3% versus 39.5% for participants on non-nucleoside reverse transcriptase inhibitors with a risk difference of 1.8% and hazard ratio of 1.09 (95% CI 0.74-1.60). Protocol deviations were non-differential across arms. In the per-protocol analysis, participants on protease inhibitors experienced treatment failure probability of 35.5% versus 29.5% in the non-nucleoside reverse transcriptase inhibitor arm for a 4-year risk difference of 6.4% and hazard ratio of 1.30 (95% CI 0.80-2.12). Shifts in failure probabilities from the intention-to-treat to per-protocol analysis were 5.7% in the protease inhibitor arm versus 10.3% in the non-nucleoside reverse transcriptase arm. Inverse probability

of censoring weights provided clinically meaningful insights into the influence of adherence on clinical outcomes. For children living with HIV in developed countries, in a setting of unknown adherence, either protease inhibitor or non-nucleoside reverse transcriptase inhibitor initial therapy may be comparable, but if adherence may be ensured, then an initial NNRTI regimen may prove superior for durable viral suppression.

B. Introduction

Adherence is critical to success of antiretroviral therapy (ART) in children living with human immunodeficiency virus (HIV). The sentinel data in adults found that a mere 10% decrease in adherence was associated with a doubling of HIV RNA viral load,[32,34] and treatment failure on ART with an unboosted protease inhibitor (PI) increased greatly with adherence <95%.[33] Continuous and optimal administration of ART decreases viral load, which decreases the probability of HIV sub-populations acquiring antiretroviral resistance mutations,[5,35] preserving the longevity of current and future ART regimens,[113] as well as decreasing chances of forward infection.[114] Unfortunately, ART appears to be less successful in producing viral suppression in children,[21] who may require more potent regimens to achieve suppression.[5] Children are also more prone to acquiring antiretroviral resistance mutations due to higher plasma viral loads, allowing more spontaneous mutations; less robust antiviral immune responses; pharmacokinetic challenges with concomitant requirements for more frequent dosing; and social and behavioral dependency.[5] Moreover, adherence challenges increase into and through adolescence, with concomitantly worse viral and immunological outcomes.[22-25,115]

Poor adherence may also complicate assessment of ART efficacy in clinical trials. Early HIV studies estimated modest average adherence around 70%,[36-39] especially among those who failed to achieve viral suppression,[40] and even successful clinical trials were only able to attain about 80% adherence.[124,125] The intention-to-treat (ITT) parameter estimates the effect of being

assigned to a treatment protocol. In standard ITT analysis of clinical trials, poor adherence typically biases estimates of effective treatments towards the null, resulting in under-estimation of treatment efficacy and over-estimation of treatment safety.[102,126,127]

The per-protocol parameter estimates the effect of being assigned to, and remaining on, a treatment protocol. Robins and Finkelstein (RF) introduced inverse probability of censoring weights (IPCW) to account for non-indicated treatment disruptions, whether by stoppage or switching of assigned therapy, or by study dropout; and describe a consistent estimator of the per-protocol effect.[95] This RF approach administratively censors study participants who cease to follow the assigned protocol, for instance, stopping or switching medications for reasons other than medical indications, such as adverse events. The approach then up-weights similar participants remaining under study to correct for induced informative censoring. The RF approach has been shown to uncover previously underpowered treatment differences,[95] correct for bias towards the null inherent in ITT analysis,[88] and estimate the effect of potentially preventable treatment discontinuations.[128] The RF provides a principled way to address problematic post-randomization protocol violations, such as poor adherence and study dropout, which may affect clinical trial results.[64]

We apply the RF approach to the PENPACT-1 (PENTA 9 / PACTG 390) trial, which randomized treatment-naïve children living with HIV-1 to ART with either a PI or a non-nucleoside reverse transcriptase inhibitor (NNRTI).[45] This trial has only previously been analyzed using ITT approaches, which yielded null results. Certain PI characteristics decrease adherence and tolerability, particularly in children: poor taste; gastrointestinal toxicity; and regimen complexity, such as pill burden, storage requirements, and dosing frequency.[18-20,37,41,60,110] However, boosted PI-based regimens appear more forgiving of treatment disruptions

than do NNRTI-based regimens.[55,57,60,96,98] We hypothesized that the null result of the ITT analysis may have resulted from a cancellation effect: Worse adherence to PIs cancelled their more robust efficacy. Therefore, we aimed to apply the RF approach to evaluate the per-protocol effect of initial PI- vs. NNRTI-based ART on time to treatment failure in treatment-naïve children living with HIV in a setting of ideal adherence.

C. Methods

C1. Study Design

PENPACT-1 (PACTG 390 / PENTA 9) was an international multicenter phase 2/3, randomized, open-label trial enrolling children infected with HIV-1 from 68 clinical centers in 13 countries in Europe and North and South America between September 25, 2002, and September 7, 2005.[45] Eligible children aged 31 days to less than 18 years were HIV-1-infected and had not received ART or only received antiretrovirals for <56 days to reduce mother-to-child transmission (excluding single-dose nevirapine). All parents or guardians and children, as appropriate, gave written consent for the parent trial; this protocol was approved by the relevant ethics committee or institutional review board (IRB) for each participating center. The per-protocol analysis was approved by IRBs at Duke University, University of North Carolina-Chapel Hill, and Children's Mercy Kansas City. This study is registered with the International Standard Randomised Controlled Trial Number Registry (ISRCTN73318385).

Children were randomized 1:1 to start ART with two nucleoside reverse transcriptase inhibitors (NRTIs) plus either a PI or an NNRTI. The study was open label, and the treating clinician chose the two NRTI drugs combined with a drug from the randomly assigned PI or NNRTI class. Children underwent clinical and HIV-1 RNA viral load assessments at randomization (week 0), weeks 2, 4, 8, 12, 16, 24, and then every 12 weeks until the last child assigned to treatment reached 4 years of follow-up (August 31, 2009). Treatment starts, changes,

and stoppages were recorded at clinical visits and *ad hoc* throughout the study, along with reasons for the medication change. Trained study personnel administered validated adherence questionnaires every 24 weeks after randomization.[61] Adherence questionnaires recorded the number of missed doses to all antiretrovirals over the 3 days prior to visit. Four years of follow-up was defined as the week 204 visit plus a 6-week lag to capture late visits. Participants were followed until their last documented viral load, viral failure, or 4 years, whichever occurred first.

C2. Protocol Deviations

Protocol deviations were defined as any non-medically indicated stoppages or changes of any component of the initially prescribed ART regimen that resulted in the participant receiving fewer than two NRTIs or not receiving the assigned class of PI or NNRTI at any time. The assigned class of PI or NNRTI on first line ART must have been according to randomized arm; the assigned class on second line ART must have been the reverse of the originally randomized class. Any switch to a third line or other regimen was considered a protocol deviation. Participants receiving both a PI and an NNRTI at the same time or use of other classes, such as integrase inhibitors or fusion inhibitors, were defined as protocol deviations, regardless of the reason for the prescription change.

ART medication stoppages or changes were captured from both treatment records and adherence questionnaires. From treatment records, we defined medically indicated reasons for ART stoppages or changes as adverse events, viral failure, resistance, death, pregnancy, regimen simplification, or medication switches within the same antiretroviral class. During the study, nelfinavir underwent a drug recall. Any immediate switch to another drug within the same class for a nelfinavir recall or other drug supply problem was defined as a non-protocol deviation, but any interruption of ART or switch to another drug class was defined as a protocol deviation, as other drugs within the same class should have been available.

Because a medically indicated reason for ART stoppage or change would require a clinician to determine the indication, any missed dose reported on the adherence questionnaire, regardless of reason, was defined as a non-medically indicated stoppage. Study dropout, defined as cessation of viral load measurements before 4 years on study, was counted as a protocol deviation. All non-medically indicated ART stoppages or changes and all dropouts were defined as protocol deviations at their first occurrence.

C3. Study Outcome

Our primary outcome was time to viral failure or clinical failure within 4 years of randomization. Time to viral failure was defined at the first of two consecutive viral loads >400 copies/mL at or after Study Week 24 (≥20 weeks after randomization). Because the protocol only required viral load confirmation of viral failure at higher viral loads (>1,000 copies/mL or >30,000 copies/mL) and other factors leading to missed viral load measurements (such as dropout, not returning for labs, and protocol deviations), many viral loads >400 copies/mL were not confirmed within a short time frame. Thus, we used the next measured viral load value, regardless of time interval, to confirm viral failure.

Clinical failure was defined as clinical disease progression, namely a new Centers for Disease Control and Prevention (CDC) stage C event (generally equivalent to a CDC stage 3 clinical event in the 2014 staging guidelines),[103,104] or other clinical progression such that the treating clinician believes that changing therapy was required prior to reaching the viral load switch point. Time to clinical failure was defined at documentation of the event in the treatment record or case report form.

C4. Covariates

Time-fixed covariates included age at enrollment (<3 vs. 3-17 years), exposure to perinatal antiretrovirals, research network/region (PACTG/IMPAACT vs. PENTA), route of

HIV exposure (vertical vs. other), baseline CDC clinical staging (stage C or not, categorized by the 1994 revised CDC classification system, as used by the parent study), baseline CD4 cell count (stage 1 vs. 2 or 3, categorized according to 2014 CDC immunologic criteria),[104,129,130] and baseline $\log_{10}$ viral load. Time-varying covariates included most recent categorized CD4 cell count from the prior visit, most recent $\log_{10}$ viral load from the prior visit, and an ART switch indicator variable. New HIV-related clinical manifestations on ART were not included because they were rare. Covariates were selected based on subject-matter knowledge and reduced to the strongest predictors of both censoring and treatment failure to ensure model convergence. The final reduced IPCW model included baseline clinical staging, baseline $\log_{10}$ viral load, time-varying categorized CD4 cell count, time-varying $\log_{10}$ viral load, and time since randomization.

Variable coding was defined according to CDC staging, trial definitions, and exploration of data fit by splines and polynomial expansion. Time since randomization was coded using a restricted quadratic spline, whereas baseline viral load and time-varying viral load fit linear and quadratic relationships on the logit scale.

C5. Statistical Analysis

We contrasted PI vs. NNRTI treatment arms as randomized in the parent study's modified ITT analysis, which included only participants who started ART. First, we performed an unweighted analysis of the original data to generate modified ITT estimates. The sole modification was removal of three participants: two who withdrew consent prior to ART initiation, and one with a major eligibility violation (Figure 5.1). Follow-up began at the date of randomization. Participants were right-censored at dropout or 4 years on study.

Then we applied IPCW to estimate the per-protocol effect. Any participant who experienced a protocol deviation was right-censored (Figure 5.1). Then, we applied IPCW to correct for imparted informative censoring. IPCW were calculated separately for each treatment

arm. For each arm, we fit a pooled logistic regression to estimate the probability of not being censored for protocol deviations using baseline and time-varying covariates for each participant at each week under study. This probability was applied as the denominator of the IPCW, and time on randomization was applied as the numerator to stabilize the weights. If time-varying values were missing, we carried forward the last observed value. Participants remaining under study were up-weighted, using the inverse of the probability of remaining free from a protocol deviation, to replace an equivalent number of participants who were censored. Censoring and up-weighting were applied cumulatively at the week of each protocol deviation until the end of 4 years on study. Similar to the modified ITT analysis, follow-up began at randomization, and participants were right-censored without up-weighting at 4 years on study.

We used descriptive statistics to characterize baseline covariates in the study sample. For the primary outcome, we estimated the risk of viral failure using the complement of the Kaplan-Meier estimator, unweighted for modified ITT analysis and applying IPCW for the per-protocol analysis. We estimated the hazard ratio (HR) for viral failure using a Cox proportional hazards model, unweighted and weighted. Proportional hazards assumptions were assessed graphically and using time interaction terms. In adjusted analyses, the ITT analysis was stratified by baseline randomized stratification variables: age, exposure to perinatal antiretrovirals, and research network (which varied by region). For the per-protocol analysis conditional on randomized stratification variables, we added the randomized stratification variables to the final reduced model for IPCW. All confidence intervals were estimated using the robust sandwich variance estimator.

Finally, we assessed the sensitivity of our results to changes in our model specifications. Given the relation between time-varying viral load from the prior visit and viral failure, an

alternative IPCW model excluded time-varying viral load. Other alternative IPCW models excluded the weakest predictor of censoring and outcome (baseline CDC clinical staging) and included only randomized stratification variables. Sensitivity analyses assessed the extremes of corrections for censoring protocol deviations by (1) estimating effects without applying IPCW; (2) assuming all censored participants in the PI arm were failures and all censored participants in the NNRTI arm were non-failures; and (3) reversing this latter coding.

Analyses were conducted in SAS® version 9.4 (Cary, NC).

D. Results

PENPACT-1 enrolled 266 HIV-1 infected children from 68 centers in 13 countries in Europe, North America, and South America. The modified ITT analysis was restricted to 263 participants who initiated ART. Participants were a median age of 6.5 years at enrollment (IQR [interquartile range], 1.8-12.9), 52% male, 49% black, and 79% exposed to HIV via vertical transmission (Table 5.1). Nineteen percent had severe clinical symptoms (CDC stage C). Median growth parameters were below average (weight-for-age Z score -0.6; height-for-age Z score -0.9). Median CD4 Z-score was -3.5, consistent with predominance of moderate to severe immunosuppression, and median viral load was 5.0 $\log_{10}$ copies/mL. Whereas 15% of children had antiretroviral exposure for prevention of mother-to-child transmission, 4% had at least one major resistance mutation at baseline. Baseline characteristics relating to mode of HIV-1 acquisition, clinical and immunological status, and ART resistance were generally balanced across ART regimens.

One hundred four participants (PI 53, NNRTI 51) experienced treatment failure by 4 years, of which 103 were viral failures and one was clinical progression despite ART. One participant died of HIV-related complications while on study but after the 4-year primary end point. In the ITT analysis, participants on PI regimens experienced a 4-year probability of

treatment failure of 41.3% vs. 39.5% for participants on NNRTI regimens for a 4-year risk difference (RD) of 1.8% and HR 1.09 (95% CI 0.74-1.60) [Figure 5.2, Table 5.2]. When stratified on randomized stratification variables, 4-year failure probabilities decreased to 34.8% and 32.6% for PI and NNRTI arms respectively, but the RD (2.2%) and HR (1.10, 95% CI 0.75-1.62) remained similar to the unadjusted ITT analysis.

Protocol deviations were frequent in the dataset. By 4 years, 118 participants were censored for any protocol deviation, of which 57 were from the PI arm and 61 were from the NNRTI arm (Table 5.3). Of these protocol deviations, 108 were due to non-medically indicated treatment disruptions (PI 55, NNRTI 53), 14 were due to dropout (PI 5, NNRTI 9), and four experienced both events in the same week (PI 3, NNRTI 1). Although non-medically indicated treatment disruptions were common and reported slightly more frequently in PI over NNRTI arms (RD 2.4%, HR 1.09 [95% CI 0.75-1.59]), dropout occurred less often in PI than NNRTI arms (RD -2.6%, HR 0.82 [0.33-2.07]) and much less frequently across arms than non-medically indicated treatment disruptions (Figure 5.3). Combining these two sources of protocol deviations yielded estimates of censoring that were generally balanced between PI and NNRTI arms (RD -2.2%, HR 0.98 [95% CI 0.68-1.40]. When stratifying on randomized stratification variables, all protocol deviation categories shifted mildly toward PI arm deviations.

In the per-protocol analysis, participants on PI regimens experienced a 4-year probability of treatment failure of 35.5% vs. 29.5% for participants on NNRTI regimens for a 4-year RD of 6.4% and HR 1.30 (95% CI 0.80-2.12) [Table 5.2]. These failure probabilities were lower than the ITT analysis, consistent with censoring of participants having protocol deviations prior to treatment failure (Figure 5.2). IPCW weights in the primary per-protocol analysis were centered near one with mean 0.981, median 0.988, minimum 0.550, maximum 1.969. When randomized

stratification variables were included in the IPCW model, 4-year failure probabilities changed to 39.4% and 30.5% for PI and NNRTI arms respectively, and RD (8.9%) and HR (1.41, 95% CI 0.85-2.35) were greater than the primary per-protocol analysis.

Shifts in treatment failure probability between ITT and per-protocol primary analyses were smaller for the PI arm than the NNRTI arm. Comparing ITT to per-protocol analyses, PI failure probability at 4 years decreased from 41.3% to 35.6% for a difference of 5.7% (Table 5.2). The ITT to per-protocol comparison in the NNRTI arm resulted in a 4-year failure probability shift from 39.5% to 29.2% for a difference of 10.3%. Cross-wise comparisons of treatment failure probabilities in the per-protocol PI arm (35.6%) versus the ITT failure probability of the NNRTI arm (39.5%) favored the PI arm by a difference of -3.9%.

Alternative model specifications did not change results substantially (Table 5.4). Sensitivity analysis estimating the probability of treatment failure in the setting of censoring for protocol deviations without IPCW correction yielded an RD of 5.1% and HR of 1.24 (95% CI 0.76-2.01) [Figure 5.4]. The extreme bound of censoring corrections that assumed all censored PI participants failed and no NNRTI participants failed yielded an RD 45.4% and HR 4.23 (95% CI 2.78-6.45). Reversing this coding for the other extreme bound yielded an RD of -40.6% and HR 0.30 (0.20-0.45). Therefore, the IPCW correction added information over censoring alone but was well within extreme bounds, suggesting that the per-protocol estimates were within reasonable limits of plausibility.

E. Discussion

Using data from the PENPACT-1 study, we generated ITT estimates followed by per-protocol estimates using the RF-IPCW approach to account for the influence of protocol deviations related to non-indicated treatment disruptions and dropouts. Consistent with the results of the parent study,[45] our ITT estimates were close to the null. However, when

administratively right-censoring for these protocol deviations and correcting for imparted informative censoring by using IPCW, the per-protocol estimates demonstrate a clinically meaningful shift in effect estimates, albeit imprecise. These PENPACT-1 per-protocol estimates are similar to those of adult clinical trials comparing ART classes that concluded regimen superiority of integrase strand transferase inhibitors (INSTIs). For example, phase 3 randomized controlled trials concluded superiority of dolutegravir-based regimens over efavirenz and ritonavir-boosted darunavir regimens at risk differences of 7%.[119,131] These results led to the elevation of INSTIs to preferred initial regimens for adolescents and adults in U. S. Department of Health and Human Services (DHHS) and World Health Organization (WHO) treatment guidelines.[132,133] Although pediatric studies are necessarily of smaller size, based on the conclusions of adult data, we suggest that the PENPACT-1 per-protocol estimates represent clinically significant results. Therefore, we suggest that pediatric PI regimens may be more robust against adherence lapses, but NNRTI regimens may have better efficacy for children in developed countries in settings of ideal adherence.

The shift between ITT and per-protocol parameter estimates lends credence to our *a priori* hypothesis that PIs are more robust against adherence lapses than NNRTIs. Non-adherence is a major driver of differential treatment failure in the PENPACT-1 trial, with a lower influence in the PI arm than the NNRTI arm. The RF-IPCW method comprises two components: (1) the relationship between treatment arm and censoring for protocol non-adherence and (2) the relationship between censoring for non-adherence and treatment failure. The relationship between treatment arm and censoring for protocol non-adherence is illustrated in Figure 5.3, where the curves for protocol deviations overlap between PI and NNRTI regimens, indicating non-differential protocol non-adherence across arms. Thus, the differential shift in treatment

failure curves from Figure 5.2A (ITT analysis) to Figure 5.2B (per-protocol analysis) derives principally from the remaining relationship between protocol non-adherence and treatment failure. The magnitude of downward shift from ITT to per-protocol curves represents failures attributable to non-adherence. These failures attributable to non-adherence were smaller in the PI arm than the NNRTI arm, with an associated smaller shift in PI (~6%) failure probabilities than NNRTIs (~10%) when estimating ITT vs. per-protocol 4-year failure risks. Since this larger shift moves the PI vs. NNRTI comparisons from essentially a null result in the ITT analysis to a clinically meaningful difference in the per-protocol analysis, we suggest that treatment failures in the PI arm were less influenced by non-adherence than NNRTIs.

Other studies comparing PI to NNRTI regimens had variable results, depending on study design, setting, and population. Similar to PENPACT-1, inadequate adherence in the NEVEREST study had less influence on viral outcomes in children continuing a ritonavir-boosted lopinavir regimen (LPV/r) than children switching to nevirapine,[99] and shifts in viral failure between the ITT and per-protocol analyses of the pediatric PROMOTE study were smaller in the LPV/r than nevirapine arm and of similar magnitude to PENPACT-1.[121] In contrast, 5-year RF-IPCW per-protocol outcomes in IMPAACT P1060 increased the rate ratio for treatment failure or death over the ITT HR in nevirapine versus to LPV/r-regimens.[84,100] Adult studies have estimated the adherence level required for viral suppression on unboosted and boosted PIs may be higher than for NNRTI regimens, but PIs are less prone to developing resistance at similar levels of adherence.[39,54,56,134-137]

As a secondary observation, the PENPACT-1 per-protocol results raise the possibility that initial NNRTI therapy may be superior to PIs for children in developed countries in the setting of ideal adherence. Although PIs and NNRTIs were comparable when non-adherence was

allowed in the ITT setting, the per-protocol analysis generated a clinically meaningful difference in viral failure under the counterfactual scenario of perfect adherence, as measured by the study. Although such a scenario is idealized, with greater accuracy in predicting adherence and better prescribing to an appropriate regimen, the more we expect that realized results would move towards the per-protocol estimate. At the extreme, perfect assignment of adherers to NNRTIs and mixed assignment of adherers and non-adherers to PIs, we might expect a 4-year contrast of the per-protocol NNRTI failure risk (29%) to the ITT PI risk (41%).

This consideration of expected outcomes under different scenarios of adherence supplements the variable results from pediatric clinical trials comparing PI and NNRTI-based regimens.[101] In IMPAACT P1060, children on LPV/r experienced less viral failure or treatment discontinuation with a 24-week risk difference of 21.5%.[100] Compared with PENPACT-1, P1060 participants were from developing countries and generally higher risk: younger ages, higher viral loads, lower CD4%, lower weight-for-age z-scores, and more advanced or severe WHO classifications. Moreover, potential reasons for LPV/r superiority may have been the higher viral loads in younger ages, with resulting greater difficulty in achieving viral suppression and higher risk of acquired resistance on nevirapine therapy, and use of half-dose strategy for the first 2 weeks of nevirapine, which may have resulted in suboptimal nevirapine concentrations at a time when viral load was most elevated. Such explanations are consistent with most nevirapine failures occurring early in the study, then stabilizing and persisting for up to 5 years afterwards.[84] In contrast, the PROMOTE study had comparable results to PENPACT-1 with Kaplan-Meier viral failure risk difference at 96 weeks of 2%.[121] PROMOTE enrolled older children up to 6 years in age with more use of efavirenz in children over 3 years old. Adult data from the Democratic Republic of Congo have also concluded similarity in treatment failure between

LPV/r and nevirapine, although a per-protocol analysis demonstrated more viral failure with nevirapine.[138]

A major difference in the PENPACT-1 data from other similar pediatric trials is the heterogeneity of ART regimens. In PENPACT-1, 62% of participants in the NNRTI arm were started on efavirenz,[45] which generally has better a better toxicity and tolerability profile than nevirapine and does not involve the ramp-up dosing phase. About half of the enrollees of the PI arm of this study were started on an unboosted PI (nelfinavir), while most of the rest were started on a boosted PI (LPV/r). Then, most participants started on nelfinavir were switched mid-study to a boosted PI because of a nelfinavir recall. Even the nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs) were heterogenous, as the treating clinicians chose the specific backbones. PENPACT-1 was designed to assess an initial treatment strategy, rather than specific regimen comparisons. However, within-class heterogeneity may have influenced results. Efavirenz-based regimens may have greater viral efficacy for children in developing countries.[139] Whereas the NEVEREST trial identified more confirmed viral failure >1,000 copies/mL for nevirapine compared to LPV/r,[99] a similar trial using efavirenz performed more favorably with lower point estimates for viral failure after switching to efavirenz and overall non-inferiority of efavirenz to LPV/r.[140] In an adult trial, LPV/r had better viral efficacy and lower resistance than nelfinavir.[58,59]

An alternative explanation of our observed ITT to per-protocol shift may be that participants on PIs experienced more medically indicated treatment disruptions that led to viral failure. Our method only censored and corrected for non-medically indicated treatment disruptions and dropouts, as medically indicated treatment disruptions, such as adverse events or pregnancy on a regimen with potential teratogenicity, would have been appropriate medical care.

If PIs and NNRTIs lead to differential medically indicated treatment switches or stoppages, which then lead to differential treatment failure, such would also lead to a separation of treatment failure curves. However, additional analysis of treatment disruptions in the PENPACT-1 data indicated that although PIs may be more difficult to tolerate, the most frequent reasons documented for treatment disruptions were events common to the classes, such as forgetting to take the medications, and overall treatment disruptions were comparable across PI and NNRTI arms.

Of note, most treatment failures occurred early in the study, most frequently by week 24. PENPACT-1 adherence questionnaires did not start until the week 24 visit, and viral failure events were only defined starting at week 24; although at 24 weeks, treatment disruptions between 0 and 24 weeks were recorded.

Finally, our strongest limitation is measurement error. Measurements of adherence were based on the study's treatment records, which note prescriptions and treatment events, and adherence questionnaires that may be prone to reporting biases. Direct measurements of drug concentrations in plasma, peripheral blood mononuclear cells, hair, or urine would have been preferable.[101,141-143]

F. Conclusion

In conclusion, reanalysis of the PENPACT-1 study using the RF-IPCW method provided clinically meaningful insights into the influence of adherence and dropout on clinical outcomes. Children living with HIV in developed countries may experience less of an influence of adherence on viral failure outcomes if assigned to initial PI therapy, but under conditions of ideal adherence, NNRTI regimens may lead to better viral outcomes. As a result, in a setting of

unknown adherence, either PI or NNRTI initial therapy may be comparable, but if adherence may be ensured, then an initial NNRTI regimen may prove superior for durable viral suppression.

Figure 5.1. Study profile for intention-to-treat and per-protocol analyses.

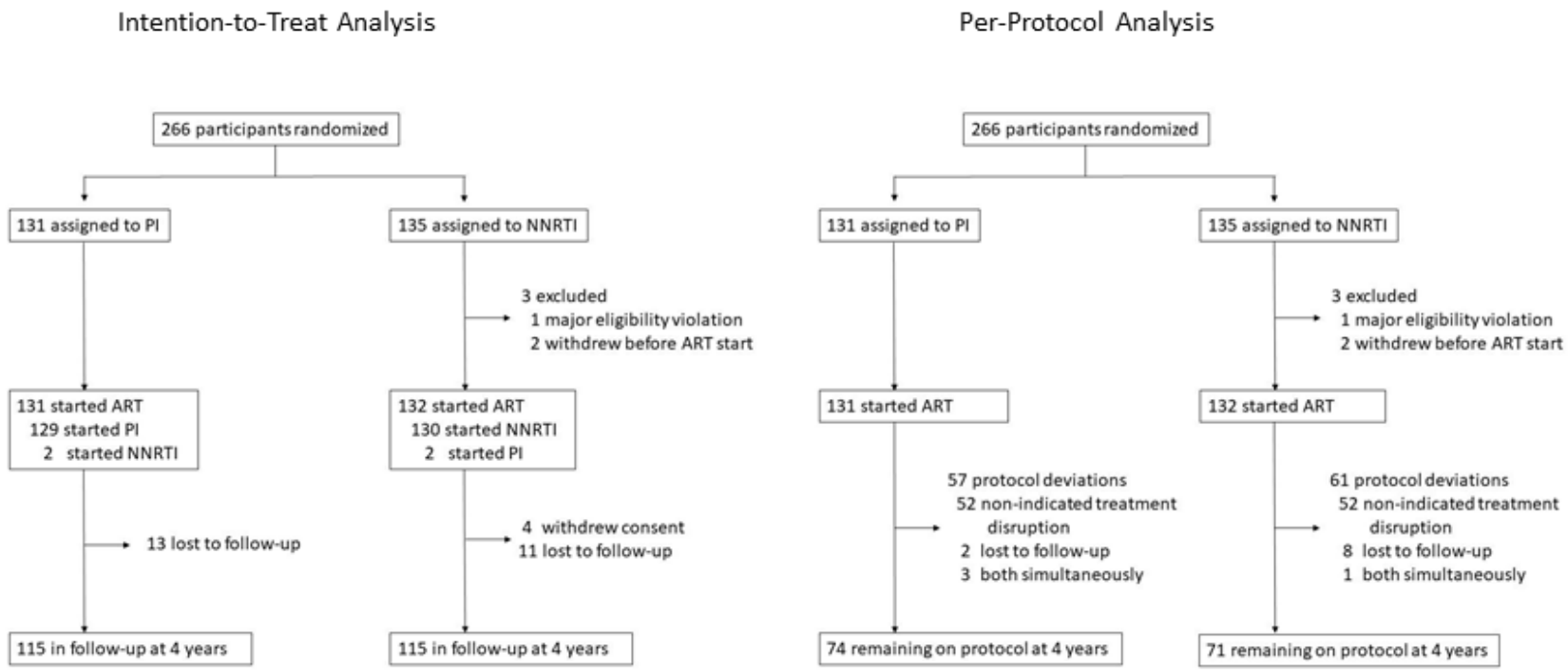


Figure 5.2. Risk of treatment failure over 4 years by treatment arm by (A) intention-to-treat analysis and (B) per-protocol analysis. The dotted line represents initial PI-based ART, and the solid line represents initial NNRTI-based ART. ART: antiretroviral therapy; NNRTI: non-nucleoside reverse transcriptase inhibitor; PI: protease inhibitor.

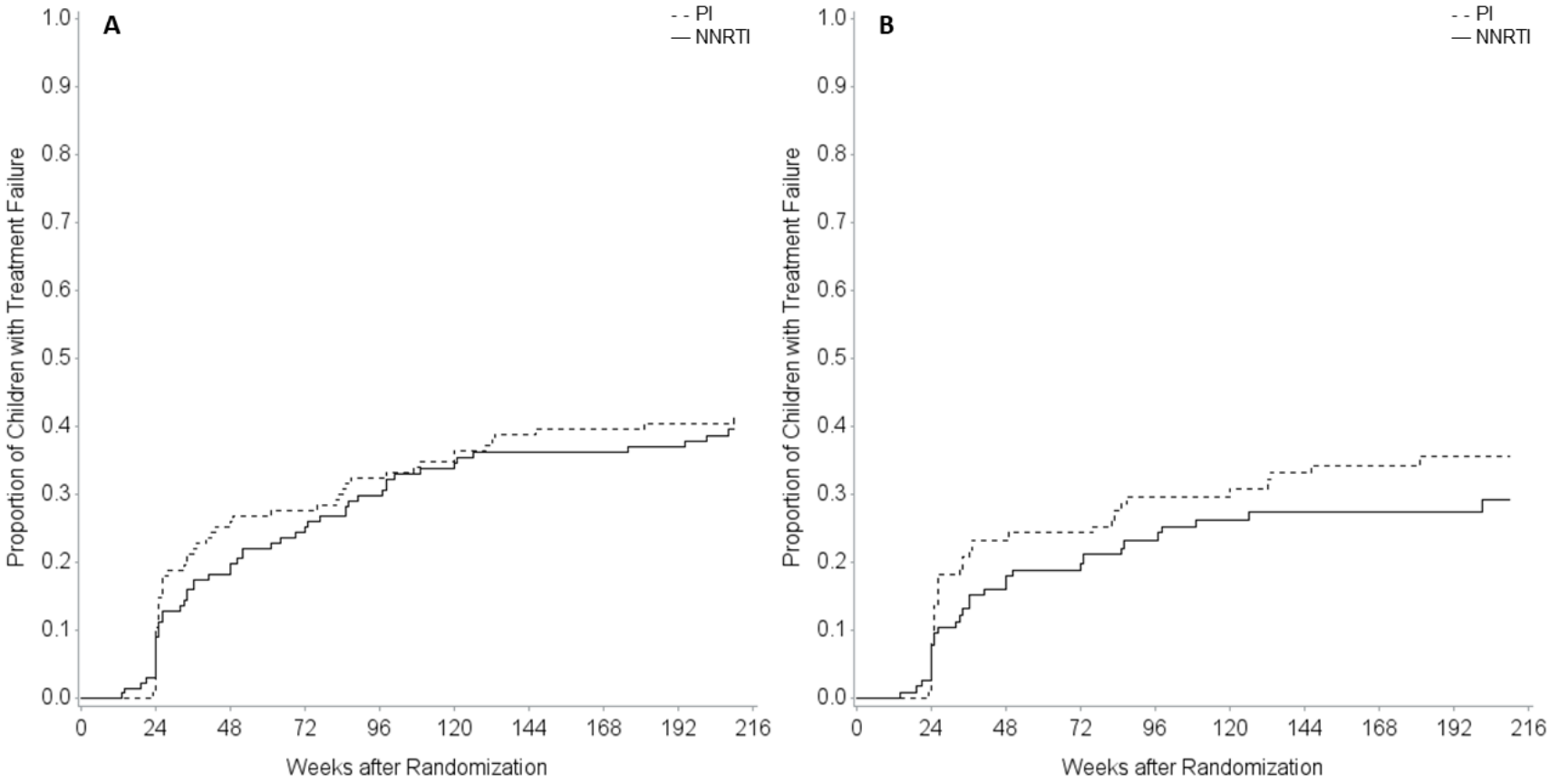


Figure 5.3. Risk of protocol deviation over 4 years by treatment arm. Panels represent proportion of children with (A) non-indicated treatment disruption, (B) dropout, and (C) any protocol deviation by 4 years, comparing initial ART with a PI vs. an NNRTI. The dotted line represents initial PI-based ART, and the solid line represents initial NNRTI-based ART. ART: antiretroviral therapy; NNRTI: non-nucleoside reverse transcriptase inhibitor; PI: protease inhibitor.

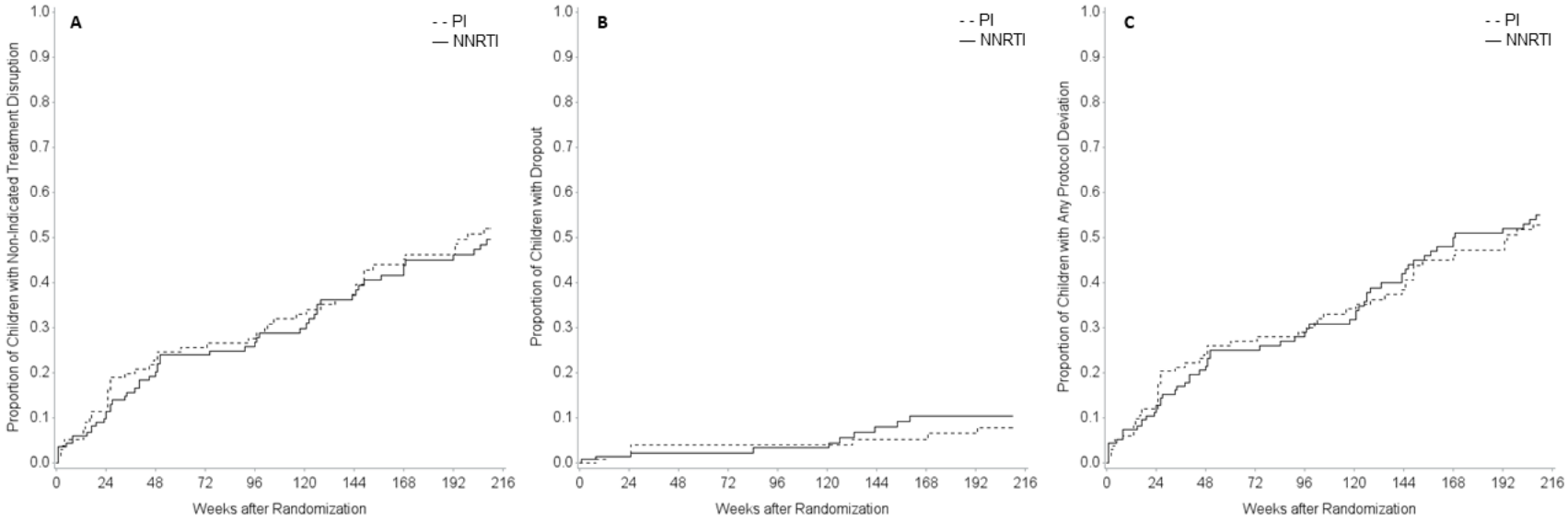


Figure 5.4. Risk of treatment failure over 4 years by treatment arm by censoring for protocol deviations without upweighting. The dotted line represents initial PI-based ART, and the solid line represents initial NNRTI-based ART. ART: antiretroviral therapy; NNRTI: non-nucleoside reverse transcriptase inhibitor; PI: protease inhibitor.

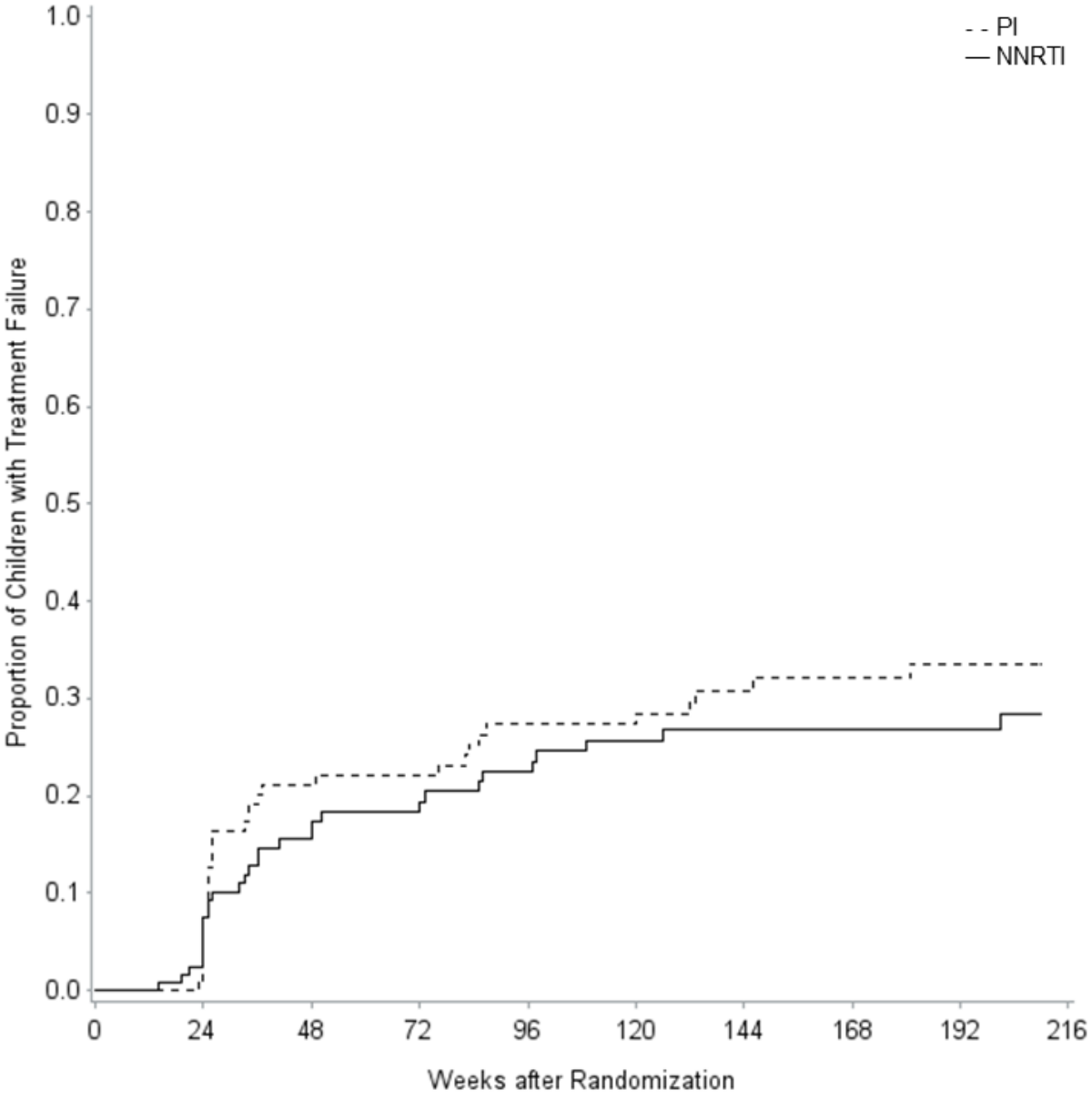


Table 5.1. Baseline characteristics of study participants according to initial ART regimen.

Variable		Randomized Group		
		PI	NNRTI	Total
N		131	132	263
Age				
<3 years	*n* (%)	34 (26%)	36 (27%)	70 (27%)
3-17 years	*n* (%)	97 (74%)	96 (73%)	193 (73%)
Age in years	Median (IQR)	7.1 (2.8, 13.7)	6.4 (2.7, 11.0)	6.5 (2.8, 12.9)
Sex				
Male	*n* (%)	69 (53%)	67 (51%)	136 (52%)
Race				
Black, Non-Hispanic	*n* (%)	60 (46%)	69 (52%)	129 (49%)
White, Non-Hispanic	*n* (%)	40 (31%)	29 (22%)	69 (26%)
Hispanic/Other	*n* (%)	31 (24%)	34 (26%)	65 (25%)
Research Network[a]				
PENTA	*n* (%)	95 (73%)	93 (70%)	188 (71%)
PACTG/IMPAACT	*n* (%)	36 (27%)	39 (30%)	75 (29%)
Route of Infection				
Vertical	*n* (%)	103 (79%)	106 (80%)	209 (79%)
Other/Unknown	*n* (%)	28 (21%)	26 (20%)	54 (21%)
CDC Clinical Stage				
N or A or B	*n* (%)	103 (79%)	109 (83%)	212 (81%)
C	*n* (%)	28 (21%)	23 (17%)	51 (19%)
Weight-for-Age Z-score	Median (IQR)	-0.5 (-1.6, 0.1)	-0.7 (-1.6, 0.2)	-0.6 (-1.6, 0.1)
Height-for-Age Z-score	Median (IQR)	-0.9 (-1.5, -0.2)	-0.9 (-1.8, 0)	-0.9 (-1.7, -0.2)
CD4 Z score	Median (IQR)	-3.6 (-7.2, -1.7)	-3.4 (-6.5, -1.4)	-3.5 (-6.8, -1.6)
Viral Load $\log_{10}$ copies/mL	Median (IQR)	5.1 (4.5, 5.7)	5.0 (4.5, 5.6)	5.0 (4.5, 5.7)
Perinatal Antiretroval Exposure	*n* (%)	19 (15%)	20 (15%)	39 (15%)
≥1 Major Resistance Mutation[b]	*n/N* (%)	5/116 (4%)	5/123 (4%)	10/239 (4%)
HIV-1 subtype				
B	*n* (%)	52 (42%)	49 (39%)	101 (41%)
C	*n* (%)	13 (11%)	12 (10%)	25 (10%)
F	*n* (%)	25 (20%)	23 (18%)	48 (19%)
A/CRF_AG/D/G	*n* (%)	21 (17%)	31 (25%)	52 (21%)
Unclassified	*n* (%)	12 (10%)	11 (9%)	23 (9%)
Switching Threshold				
1,000 copies/mL	*n* (%)	66 (50%)	68 (52%)	134 (51%)
30,000 copies/mL	*n* (%)	65 (50%)	64 (48%)	129 (49%)

Abbreviations: ART, antiretroviral therapy; IQR, interquartile range; *N*, total sample size; *n*, subsample size; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor.

[a] PENTA sites were predominantly in Europe, South America, and the Bahamas. PACTG/IMPAACT sites were based primarily in the United States.

[b] Not all patients had successful baseline genotypic resistance assays.

Table 5.2. Hazard ratios of treatment failure comparing initial PI- vs. NNRTI-based regimens.

Analysis	PI Failure Probability at 4 years (%)	NNRTI Failure Probability at 4 years (%)	Risk Difference at 4 years	Hazard Ratio (95% CI)
Primary Analyses				
Intention-to-treat (ITT) analysis	41.3%	39.5%	1.8%	1.09 (0.74-1.60)
Per-protocol (PP) analysis	35.6%	29.2%	6.4%	1.30 (0.80-2.12)
Conditional on Randomized Stratification Variables				
ITT with stratification on randomized stratification variables	34.8%	32.6%	2.2%	1.10 (0.75-1.62)
PP with censoring and IPCW including randomized stratification variables	39.4%	30.5%	8.9%	1.41 (0.85-2.35)

Abbreviations: CI=confidence interval; IPCW=inverse-probability of censoring weights; ITT=intention-to-treat; NNRTI=non-nucleoside reverse transcriptase inhibitor-based regimen; PI=protease inhibitor-based regimen; PP=per-protocol.

Table 5.3. Hazard ratios of protocol deviations or dropout by initial PI- vs. NNRTI-based regimens.

Analysis	PI Probability at 4 years (%)	NNRTI Probability at 4 years (%)	Risk Difference (%)	Unstratified Hazard Ratio (95% CI)	Stratified Hazard Ratio (95% CI)
Non-Medically Indicated Treatment Disruptions	52.0%	49.7%	2.4%	1.09 (0.75-1.59)	1.21 (0.84-1.76)
Dropout	7.8%	10.4%	-2.6%	0.82 (0.33-2.07)	0.86 (0.35-2.13)
Any Protocol Deviation (Non-Indicated Disruption or Dropout)	52.9%	55.1%	-2.2%	0.98 (0.68-1.40)	1.09 (0.76-1.57)

Abbreviations: CI=confidence interval; NNRTI=non-nucleoside reverse transcriptase inhibitor-based regimen; PI=protease inhibitor-based regimen; PP=per-protocol.

Table 5.4. Alternative model specifications and sensitivity analyses.

Analysis	PI Failure Probability at 4 years (%)	NNRTI Failure Probability at 4 years (%)	Risk Difference at 4 years	Hazard Ratio (95% CI)
Alternative Models				
PP with censoring for protocol deviations and IPCW model without time-varying viral load	33.7%	28.6%	5.2%	1.25 (0.77-2.03)
PP with censoring for protocol deviations and IPCW model without time-varying viral load and baseline clinical stage	34.2%	28.5%	5.7%	1.26 (0.77-2.05)
PP with censoring for protocol deviations and IPCW model with time-varying viral load without baseline clinical stage	36.0%	29.4%	6.6%	1.30 (0.80-2.12)
PP with censoring for protocol deviations with IPCW using only randomized stratification variables and time on study	34.0%	29.4%	4.6%	1.22 (0.74-2.00)
Sensitivity Analyses				
PP with censoring for protocol deviations (without IPCW)	33.5%	28.4%	5.1%	1.24 (0.76-2.01)
PP with censoring and single imputation (all censored PIs as failures and all censored NNRTIs as non-failures)	68.0%	22.6%	45.4%	4.23 (2.78-6.45)
PP with censoring and single imputation (all censored PIs as non-failures and all censored NNRTIs as failures)	26.4%	67.0%	-40.6%	0.30 (0.20-0.45)

Abbreviations: CI=confidence interval; IPCW=inverse-probability of censoring weights; NNRTI=non-nucleoside reverse transcriptase inhibitor-based regimen; PI=protease inhibitor-based regimen; PP=per-protocol.

CHAPTER 6: CONCLUSION

A. Overview

We aimed to examine the effects of treatment regimen, protocol non-adherence, and treatment failure in our analysis of the PENPACT-1 trial. Although the parent trial found a null effect of initial PI vs. NNRTI-based ART, we hypothesized that this null result was due to canceling effects of protocol non-adherence and regimen potency. Specifically, we hypothesized that PIs would have worse time to treatment disruption but be more robust against treatment failure in the setting of protocol non-adherence. Our hypothesis was wrong.

In Aim 1, we assessed randomized initial PI vs NNRTI-based ART in children for differences in treatment disruption at 4 years after randomization and end of study. PIs and NNRTIs had similar time to treatment disruption, although the PI class appeared to result in greater reported intolerance. Despite this greater intolerance, the most common reasons for treatment disruption were common to both regimens, such as forgetting to take the medications, and participants persisted in ingesting the both regimens despite differences in tolerability.

In Aim 2, we performed an ITT analysis of initial PI vs. NNRTI-based ART on time to treatment failure by 4 years after randomization. Then, we performed a per-protocol analysis applying the RF-IPCW method to administratively right-censor participants at the time they experienced a protocol deviation, then upweight similar participants remaining in the study to correct for possible imparted informative censoring. The ITT analysis yielded estimates close to the null. The per-protocol analysis yielded estimates favoring initial NNRTI-based ART with lower 4-year treatment failure probability, indicating possibly better potency of initial NNRTI-

based therapy in the setting of ideal protocol adherence. Further, initial PI-based ART had a smaller shift in estimates from the ITT to per-protocol estimates, indicating greater robustness against the effects of protocol non-adherence.

Thus, our overarching hypothesis was disproven, and the explanation was contrary to our initial reasoning. Treatment disruption was similar between arms, meaning that that protocol non-adherence was not a differential contributor to the regimen effects under study. Instead, the cancelation effect observed in PENPACT-1 appears to result from canceling effects of regimen potency and robustness. Specifically, NNRTI-based regimens appear to have greater treatment potency (lower treatment failure probability) than PI-based ART in the setting of ideal ART protocol adherence, but NNRTI's appear to be more prone to influences of non-adherence. Because non-adherence led to a greater shift in treatment efficacy in NNRTIs than in PIs, the NNRTI treatment failure curved moved more than the PI curve in the setting permissive of non-adherence such that the treatment failure curves equalized. In other words, regimen potency and robustness canceled.

A1. Aim 1 Summary of Key Findings

In Aim 1, we investigated the relationship between treatment regimen and time to treatment disruption. Our essential question was whether prescribing a PI or NNRTI-based initial ART would affect the time a child may maintain optimal, uninterrupted first-line therapy. To this aim, we analyzed the PENPACT-1 data, which randomized children aged 31 days to <18 years to initial ART with two NRTIs plus a PI or an NNRTI. We analyzed the same modified ITT population as the parent trial to assess time to the participant's first treatment disruption event, using participant treatment records and adherence questionnaires. Participants were followed until the primary study endpoint of 4 years after randomization and at end of study. We analyzed the data using the complement of the Kaplan-Meier and Cox proportional hazards models.

The large majority of participants (>70%) experienced at least one treatment disruption during the study. At 4 years, 7% more participants randomized to initial PI-based regimens experienced a treatment disruption event than participants randomized to initial NNRTI-based ART. By the end of study, differences in time to treatment converged. Overall hazard ratios were close to the null, and confidence intervals were wide given the modest study size.

On exploration of reasons for treatment disruption, PI regimens appeared to have greater problems with tolerability, such as adverse events, problems taking the medications, and refusal to take the drugs, as well as scheduling/lifestyle interference. Nevertheless, the most frequently reported reasons for non-adherence on the questionnaire were common reasons in both arms, such as forgetting/lack of support, and running out of drug.

In summary, children frequently experience treatment disruptions to ART, but time to treatment disruption was similar for initial PI- and NNRTI-based ART. Although PI regimens appear to have greater reported tolerability problems, participants persisted in taking both regimens similarly. Initial ART with either a PI or NNRTI may be acceptable for maintaining optimal, continuous therapy.

A2. Aim 2 Summary of Key Findings

In Aim 2, we delved further into the relationship between protocol non-adherence and treatment failure. As per Aim 1, we studied treatment-naïve children living with HIV-1 in developed countries from the PENPACT-1 trial. We aimed to estimate ITT and per-protocol effects of initial PI versus NNRTI-based ART on time to treatment failure, defined as viral or clinical failure, by 4 years after randomization. By contrasting the ITT and per-protocol estimates, across and within treatment arms, we evaluated the shift in ITT to per-protocol estimates for PI regimens versus NNRTI regimens, with the difference in ITT to per-protocol estimates defining “robustness” to the nontrivial amounts of non-adherence.

We first performed a modified ITT analysis, as per the definitions of the parent trial. This ITT analysis generated an estimate of the effect of ART regimen on time to treatment failure in a setting permissive of protocol non-adherence.

Then, we performed a per-protocol estimate by applying the RF-IPCW method. In contrast to the definitions of any treatment disruptions in Aim 1, we defined only treatment disruptions that were not medically indicated as protocol deviations. By differentiating between medically indicated treatment disruptions and non-medically indicated disruptions, we allowed for stops or changes in therapy for adverse events or other appropriate care. Dropout was also defined as a protocol deviation. Once a participant experienced a non-medically indicated treatment disruption or dropout, the participant was administratively right censored. To account for informative censoring, we constructed IPCW based on time-fixed and time-varying covariates to upweight similar participants remaining on study. This per-protocol analysis generated an estimate of the effect of ART regimen on time to treatment failure in the setting of ideal protocol adherence.

Time to treatment failure was estimated using the complement of the Kaplan-Meier estimator and Cox proportional hazards models. IPCW were estimated using pooled logistic regression models and stabilized using weeks since randomization.

We estimated the ITT parameter, the risk of protocol deviations, and the per-protocol parameter. As per the parent trial, the ITT estimate had a risk difference of 1.8% with a hazard ratio close to the null. Although protocol deviations were common, they were non-differential by PI vs. NNRTI treatment arm. In the per-protocol analysis, the 4-year risk differences expanded to 6.4% unadjusted and 8.9% adjusted, with greater hazard ratios favoring NNRTI-based regimens, albeit imprecise. Notably, the shifts in ITT to per-protocol estimates were smaller for PIs (~6%)

than for NNRTIs (~10%), consistent with PIs being more robust against protocol non-adherence.

In summary, in a setting permissive of protocol non-adherence, initial PI vs. NNRTI-based ART had similar time to treatment failure. However, in a setting of ideal protocol adherence, NNRTI-based ART appear to have better point estimates for treatment efficacy. The lack of differences in protocol deviations do not support protocol adherence as a differential driver of treatment failure. Instead, the greater regimen potency for NNRTIs and the greater robustness of PIs against protocol non-adherence appear to be drivers of cancelation effect, resulting in the null effect observed in the ITT analysis.

B. Strengths

The strengths of this work lie in the structure of the data, the causal inference methods applied, and the bridge created by linking randomized clinical data to observational data analysis using a principled approach.

This project used data from the PENPACT-1 trial, the first randomized clinical trial in children comparing with two different classes of ART.[45] By analyzing these data, we have generated one of the first randomized comparisons of pediatric adherence to specific ART classes. These data also followed participants longitudinally for an extended follow-up period until 4 years after the last enrollee and up to 6.5 years for some participants. This duration of follow-up, along with the broad study population base on three continents in 13 countries at 68 sites across two pediatric HIV networks, stood as one of the largest and richest datasets of its time.

Inherent within the design of this randomized clinical trial was the fulfillment of many criteria for causal identification.[144,145] Randomization ensured exchangeability of the treatment groups, conditional on the randomized stratification variables. Further the data structure supported positivity in both aims. In Aim 1, assessment of treatment disruption across

randomized arms ensured positivity, as all analyses were performed across measured strata of treatment arm, stratification variables, and outcome measurements. In Aim 2, we identified no inflation of IPCW to suggest any positivity violations. Finally, the randomized structure allowed general consistency in contrasts of PI vs. NNRTI ART classes, but the heterogeneity of specific drug choices within classes may have compromised the assumption of treatment invariance, which is discussed further in the limitations below.

While the data structure supported claims to causal inference for ITT analyses, the structure of the trial data also lent naturally to observational data analysis of post-randomization factors.[64] In Aim 2, our focus was on the influence of post-randomization factors of protocol deviations, primarily related to non-medically indicated treatment disruptions and dropout. Because the initial treatment intervention was randomized with longitudinal follow-up, our per-protocol analysis could better isolate influences of these post-randomization factors. In other words, our analysis leveraged the trial's randomized design to allow valid examination of post-randomization effects through causal inference methods.

The strengths of the methodology may be illustrated by comparisons to other per-protocol analysis approaches.[64] In a traditional per-protocol analysis, the analyst determines which participants experienced a protocol deviation, excludes such participants, and performs the analysis with only the participants who remained adherent to the protocol until the study's primary end point. Traditional per-protocol methods introduce problems, including limiting sample size and failing to account for post-randomization informative censoring. For traditional per-protocol estimates to generate unbiased estimates, one must assume that participants who underwent protocol deviations were similar to participants who remained adherent to the protocol, namely that right-censoring is non-informative. This is an unduly strong assumption.

Indeed, the very fact that some participants underwent protocol deviations while others did not stands as evidence that this assumption is likely untrue and thereby jeopardizes exchangeability of these groups. In addition, the exclusion of participants who remained adherent to the protocol limits the sample size and discards information available about participants before the protocol deviation. This exclusion of data also hampers the per-protocol parameter's precision. Thus, the traditional per-protocol approach tends to bias estimates of both the per-protocol parameter and its variance.

Our Aim 2 analysis estimated the per-protocol effect by using the RF-IPCW.[95] Similar to a traditional per-protocol analysis, this approach detects protocol deviations and drops data. In contrast to traditional per-protocol analysis, the RF-IPCW approach does not drop all data from a participant experiencing a protocol deviation but only right-censors at the time when the protocol deviation is detected. Then, by using modeling to approximate conditional exchangeability between censored participants and similar remaining participants, IPCW upweights the similar remaining participants to compensate for informative censoring. Applying stabilized weights helps restore a similar original sample size, thereby maintaining a closer approximation to the original sample size and retaining precision. This approach removes the bias due to informative censoring if a correct set of time-varying covariates is accounted for, but the extra variability added by the estimated weights further reduces precision (in exchange for the possible bias reduction).

Another per-protocol approach that bears similarity to RF-IPCW directs clinical trial enrollment and analytical decisions on participant evaluability. In this approach, study participants are assessed during the trial for meeting certain procedural milestones, such as remaining adherent to the prescribed regimen and continuing follow-up evaluations until a pre-

specified trial time point. If not all procedural milestones are met, then this participant and her data may be removed from the trial. Then other potential enrollees would be screened, and if eligible, another participant would be enrolled to replace the removed participant. Although similar in concept, the RF-IPCW method provides better compensation for informative censoring, as upweighting a sub-population of similar remaining participants better approximates the outcome of the removed participant than a single random replacement, and obviates the potential pragmatic, safety, and cost concerns of the replacement method. Thus, RF-IPCW may provide a more valid and pragmatic method for informative censoring correction than even real-time replacement methods.

C. Limitations

The principal scientific question of the parent study was to investigate the viral effects of initial classes, namely PI vs. NNRTIs. This strategy has been rather unique among pediatric RCTs as a class-wide comparison of initial regimens with allowances for switching regimens when clinically indicated. As a result, the principal comparisons include heterogeneity among specific drugs within classes and switching the initial regimens based on two different pre-specified viral criteria. The most relevant comparative pediatric trials contrasted specific drugs in each class, usually LPV/r versus nevirapine with or without specification of accompanying NRTI backbones and without the same freedom to switch drugs for the same viral outcome. These trials had mixed results, with one major trial supporting PENPACT-1 trial results and another with strong superiority of LPV/r over nevirapine.[100,121] These trials differed in study populations from PENPACT-1, as most participants in those trials were younger, possibly sicker, and in developing countries, but a major difference was in their homogeneity of treatment contrasts. PENPACT-1 had more heterogeneity of regimens with unboosted and boosted PIs, nevirapine or efavirenz for the NNRTI, and heterogeneity in the NRTI backbone. Although PENPACT-1 was

intended to evaluate ART classes, variability of specific drug choices may compromise the assumption of treatment invariance.

Another major limitation was measurement error. First, we had no direct measures of drug exposure, such as therapeutic drug monitoring. Treatment records captured only prescribing events and documented ART disruptions, and the adherence questionnaires relied on accurate reporting by either the child or the caregiver, if present and willing to answer. Although we relied on a questionnaire that has previously been validated,[61] reporting biases and unanswered questionnaires may have affected our measures of missed doses. Direct measurements of drug concentrations in plasma, peripheral blood mononuclear cells, hair, or urine would have been preferable.[141-143] Second, adherence questionnaires in this study focused on ART adherence over the 3 days prior to the most recent visit and inquired about adherence barriers encountered over the prior 2 weeks, rather than a daily measure of adherence throughout the study. The time-varying nature of treatment disruption means that patients may have experienced an initial or temporary period of treatment disruption that was subsequently corrected, but our analysis presents only data on time to first event of treatment disruption. Of note, most treatment failures occurred early in the study, most frequently by week 24. PENPACT-1 adherence questionnaires did not start until the week 24 visit, and viral failure events were only defined starting at week 24; although at 24 weeks, treatment disruptions between 0 and 24 weeks were recorded. Third, limited participant report of individual drugs missed on the adherence questionnaire precluded definitive identification of treatment disruptions of individual drugs. Instead, we assessed treatment disruption to any component of the ART regimen. Fourth, heterogeneity of adherence questionnaires across networks, ages, and respondents regarding barriers to therapy should caution against rigorous interpretation of reasons for treatment disruptions.

In our interpretation of the potential drivers of the cancelation effect, we must recognize that other explanations of the data are plausible. An alternative explanation of our observed ITT to per-protocol shift may be that participants on PIs experienced more medically indicated treatment disruptions that led to viral failure. Our method only censored and corrected for non-medically indicated treatment disruptions and dropouts, as medically indicated treatment disruptions, such as adverse events or pregnancy on a regimen with potential teratogenicity, would have been appropriate medical care. If PIs and NNRTIs led to differential medically indicated treatment switches or stoppages, which then led to differential treatment failure, such would also have led to a separation of treatment failure curves. However, additional analysis of treatment disruptions in the PENPACT-1 data indicated that although PIs may be more difficult to tolerate, the most frequent reasons documented for treatment disruptions were events common to the classes, such as forgetting to take the medications, and overall treatment disruptions were comparable across PI and NNRTI arms.

Finally, this study size limited the precision of our estimates. In Aim 1, the size was not sufficient to distinguish differences on the order of 7%, as was seen at 4 years. In Aim 2, confidence intervals were wide relative to the effect estimates, despite clinically meaningful shifts in ITT to per-protocol effect estimates.

D. Public Health Significance and Conclusion

The major insights from our project relate to subject matter, methodology, and a new understanding of the potential components driving observed treatment efficacy.

Regarding subject matter, we conclude that NNRTIs may not be inferior initial ART for children in developed countries, particularly in a setting of low NNRTI resistance and expected good adherence to ART. Based on another clinical trial finding superiority of LPV/r over nevirapine-based ART in children <3 years old in developing countries, the WHO has favored

LPV/r as initial therapy for children under 3 years old.[84,100,146] Resistance to NNRTIs has also been increasing in developing countries, where nevirapine is still commonly used for prevention of mother-to-child transmission.[147] In our PENPACT-1 analyses, time to treatment disruption and time to treatment failure were comparable for NNRTIs and PIs in settings permissive of protocol non-adherence. In a setting of ideal protocol adherence, point estimates favored initial NNRTI-based ART. Since the PENPACT-1 protocol screened for baseline resistance as part of eligibility criteria, our study population had low levels of resistance. In this setting, the US DHHS guidelines for pediatric ART continue to prefer initial ART with two NRTIs and either a PI, NNRTI, or INSTI.[101] We suggest that for children in developing countries without baseline NNRTI resistance, initial NNRTI-based ART remains a viable option, especially if adherence may be ensured.

Methodologically, we have demonstrated the flexibility and utility of the RF-IPCW approach as applied to a pediatric HIV clinical trial. To our knowledge, we are the first to highlight the shift in risk of outcomes within a treatment across the ITT and per-protocol parameters. Highlighting this within-treatment arm difference may be helpful in understanding the effects of treatment, protocol adherence, and robustness to protocol non-adherence. Post-randomization effects, particularly non-adherence, remain major determinants of HIV outcomes and may be influential on ITT outcomes.[64,95] RF-IPCW have not yet seen demonstrable uptake in pediatric HIV clinical trials.[84] In this case, RF-IPCW revealed insights into the relationships among treatment regimen, protocol non-adherence, regimen potency, and regimen robustness in contributing to observed treatment efficacy. Our results disproved our own hypothesis that regimen-specific non-adherence was a major component of the observed cancelation effect, and the drivers of the observed null effect were only identified by examining effects through the RF-

IPCW method.

Finally, we conclude that a new framework for understanding the components of treatment efficacy may be needed. Rather than non-adherence canceling the efficacy of the treatment regimens, as we had hypothesized, regimen potency and robustness canceled. Instead of focusing on the direct effects of non-adherence on treatment outcomes, perhaps we should conceptualize regimen potency versus robustness. Throughout this project, we conflated both these concepts into one concept of "treatment efficacy". Some adult adherence literature has already delineated some aspects of treatment goals. For example, a study in adults evaluated whether ART regimens require different adherence thresholds for viral suppression versus avoidance of antiretroviral resistance.[54] We submit that the literature may not have gone far enough. Most publications do not explicitly separate out regimen potency and robustness as components of treatment efficacy. In our analysis of PENPACT-1, regimen potency and robustness were opposing effects and canceled sufficiently to yield a null effect estimate in the ITT analysis. However, these effects may not always oppose, nor may their magnitudes always be similar enough to cancel. We conclude that considering regimen potency and regimen robustness, in addition to traditional components (*e.g.*, adherence), would provide a more detailed framework for the various elements contributing to the composite outcome called treatment efficacy.

APPENDIX 1: ADDITIONAL BACKGROUND ON ADHERENCE IN HIV-INFECTED CHILDREN

A. Challenges to Pediatric ART Adherence

Pediatric adherence is a challenge. Adherence challenges are accentuated in pediatric populations,[22-25] as pediatric adherence is directly related to the overall health and psychosocial factors of the entire family. Poverty and low caregiver education, along with the resultant shortages in nutrition and transportation, have been associated with worse adherence in children.[19,42,47,49,50,76,148] In addition, health and financial strains may impact adherence by weakening the caregiver-child relationship.[76] At the extreme, many children are left as orphans, who are especially prone to poor adherence.[42,149] While some children are more adherent under the care of foster parents than biological parents,[44] altered support systems add their own complexities. Impaired care support structures highlight pediatric adherence struggles.

Unique to pediatric adherence are the rapid transitions across ages. Although published literature has not been consistent regarding age-related effects for younger children,[43,44] the dependency and needs of pediatric patients differ widely as they grow from infancy to adolescence.[22-25] Adherence concerns move from simple child refusal when younger[20] to lack of commitment when adolescents.[24] As age increases, fear of stigma also shifts from being an exclusive parental concern to becoming a peer-pressure driven individual concern. Stigma can strongly influence adherence, although the direction of the influence may be in either direction.[47,49,150] Nevertheless, an overarching age-dependent adherence consideration is degree of supervision.[20,76] Caregiver factors may explain why some studies conflict with prior data on adherence effects of income and degree of childhood illness.[44,77] Finally, age-dependent factors must also consider treatment fatigue.[42,47] Supporting adherence from earlier ages or through difficult periods may become progressively more difficult over time, and lifelong treatment is

longer in children than in adults. With unique challenges in pediatric populations, one must identify factors that are modifiable and sustainable.

B. Potentially Modifiable Risk Factors for Poor Adherence to ART in HIV-Infected Children

Some reasons for poor adherence relate to simple issues of daily life (Table S1). Adherence questionnaires find that reasons for missing doses include child refusal[20] and forgetting.[76] Daily routine appears to play a role, as disruptions of routine or scheduling issues are reported adherence barriers.[20,77]

Many families fear disclosure of HIV status to their children, thereby precluding children's involvement in their own care.[20,50,71,151] Lack of disclosure has been consistently associated with worse adherence.[24,49,77,148,151-153] Disclosure also has benefits for mental health, psychosocial development, caregiver well-being, and future planning,[152] while not being associated with emotional trauma or divulging to others.[153] Children who know their HIV status have less frustration, less conflict in the child-caregiver relationship, and less conflict about medications.[76] However, caregivers struggle with acceptance of the HIV diagnosis themselves and fears of disclosure.[154] Thus, disclosure is a major adherence issue that should be addressed at the caregiver level.

Optimal pediatric adherence depends on caregiver involvement. Caregiver supervision is critical,[20,76] and caregiver mental health—especially substance abuse and depression—plays a major role in pediatric adherence.[47,50,87] Caregiver substance abuse is commonly identified as an adherence challenge.[50,87] Depression is also common and negatively impacts ART adherence,[47] particularly in the postpartum period.[155] Indeed, a caregiver's well-being is affected by the HIV diagnosis.[154] Support for caregivers can benefit not only the caregiver, but also the child's adherence.[71,76] Thus, services directed at caregivers may optimize pediatric care.

Mental health challenges in children also are a major barrier to antiretroviral adherence. HIV-infected children in particular suffer from psychiatric diagnoses, such as depression, anxiety, disruptive disorders, hyperactive disorders, and post-traumatic stress disorder[156-158]— which require more intensive psychiatric interventions.

To date, most pediatric adherence interventions have focused on social and behavioral interventions.[159] At the level of the individual child, children who have received education about ART,[24] pill swallowing training,[89] or disclosure of HIV status have had better adherence.[24,49,77,148,151-153] Tools such as use of pill carriers may be beneficial,[24] but medication reminders have had mixed results.[41,49] At the caregiver level, adherence education and counseling have been associated with better adherence.[49,87,160] Home-based therapy also yields improvements.[41,160-163]

However, little research has been published on physician-level decisions about treatment regimens to optimize adherence.

Table S1. Potentially modifiable risk factors for poor pediatric adherence.

Risk Factors
Dysfunctional Family System: • Forgetfulness • Medication refusal • Disrupted household routine • Lack of child supervision • Lack of support for caregiver
Lack of disclosure of HIV status to child
Child and caregiver mental health diagnosis
Drug regimen

APPENDIX 2: ADHERENCE QUESTIONNAIRES USED AT PACTG SITES

09-09-04
QL5002(390)/07-22-02

PEDIATRIC ADHERENCE QUESTIONNAIRE MODULE 1- REVISED

Behavior/Identification

NIAID PEDIATRIC AIDS CLINICAL TRIALS GROUP

Patient Number [ ][ ][ ] [ ][ ][ ][ ] [ ] Date of Patient Visit [ ][ ][ ] [ ][ ] [ ][ ][ ][ ]
mmm dd yyyy

Protocol Number [3][9][0][ ][ ][ ]

Institution Code [ ][ ][ ][ ][ ][ ]

Form Week [ ][ ][ ] * Seq No. [ ] **Step No. [ ][ ] Key Operator Code [ ][ ][ ]

* Enter a "1" if this is the first of this form for this date. Designate subsequent forms on the same date with a 2, 3, etc.
**Enter the subject's current study step number. Enter '1' if the study does not have multiple steps.

The **purpose** of this questionnaire is to collect data which will quantify the proportion of prescribed antiretroviral therapy actually taken within the last 3 days in order to examine drug effect during the analysis phase of a study protocol. In order to have confidence in the data that will be collected with this instrument, the **process** through which these data are collected has been **standardized**. Please read and observe the following directions.

Who administers? Any member of the professional clinical care or research team who has experience filling out other ACTG study forms.

Who is questioned? The research subject, if the subject has assumed responsibility for his/her own drug regimen OR the subject's primary caregiver (the person responsible for administering the prescribed drugs at home). If the appropriate respondent is the primary caregiver and she/he is not present for a particular study visit when this form is scheduled, the form should be marked as such (below) and the assessment omitted.

How is the questionnaire administered? The questionnaire has very specific introductory comments (and prompts) which should be read as stated, item after item, until the assessment is completed. No interruptions for subject education should occur; any necessary or additional education or counseling should take place **after** the questionnaire is completed.

The accuracy of self report is very good if the attitude of the interviewer is non-judgemental and supportive. The form has introductory statements to set this tone. However, since information about erratic adherence may be elicited by the survey, what interaction happens after the questionnaire is completed is critically important. The attitude of the clinical response, the manner in which information is subsequently corrected, and the nature of behavioral counseling will **absolutely** influence future validity. It is imperative that clinical teams review the characteristics of therapeutic relationships and strive to adopt these practices in promoting drug adherence in study subjects.

QUESTIONS 1 - 3 ARE TO BE COMPLETED BY THE STUDY NURSE:

1. Was the questionnaire completed at this visit? ……..(1-Yes, 2-No) [ ]
   If Yes, go to question 2.
   If No, complete 'a' and STOP.

   a. Indicate the reason the questionnaire was not completed:…… [ ]
   1-Subject refused
   2-Primary caregiver refused
   3-Subject missed clinic visit
   4-There was not enough time
   5-Primary caregiver not available
   9-Other reason, specify

   If Other reason, specify [30]: _____

2. Who responded to the questions?……1-Subject [ ]
   2-Biological mother
   3-Biological father
   4-Other relative, specify
   5-Adoptive parent
   6-Foster parent
   9-Other, specify

   If "4-Other relative" or "9-Other", specify [30]: _____

12-13-99/03-27-00/07-05-01

Pt. No. ☐☐☐ ☐☐☐☐ ☐ * Seq. No. ☐ ** Step No. ☐☐ Date ☐☐☐ ☐☐ ☐☐☐☐
mmm dd yyyy

3. Since the last visit, did the subject utilize any of the following aids for improving adherence?..... (1-Yes, 2-No) ☐
   If No, go to the Medication Table.
   If Yes, answer 'Yes', 'No' or 'Not known' to each of the following:

(1-Yes, 2-No, 3-Not known)

a. Labels: ..... ☐
b. Calendars: ..... ☐
c. Pill boxes: ..... ☐
d. Beepers: ..... ☐
e. Monitoring caps (MEMS): ..... ☐
f. Timers: ..... ☐
g. Programmable wrist watches: ..... ☐
h. Diary: ..... ☐
i. "Buddy system": ..... ☐
j. PEG/gastrostomy tube:..... ☐
k. Activity of daily living triggers, specify: ..... ☐

   Specify[30]: _____

l. Other, specify: ..... ☐

   Specify[30]: _____

12-13-99/03-27-00/07-05-01

PEDIATRIC ADHERENCE QUESTIONNAIRE MODULE 1 - REVISED

Pt. No. ☐☐☐ ☐☐☐☐ ☐ * Seq. No. ☐ ** Step No. ☐☐ Date ☐☐☐ ☐☐ ☐☐☐☐
mmm dd yyyy

INSTRUCTIONS FOR COMPLETION OF MEDICATION TABLE

- **Columns A-D:** Prior to the study visit, the study nurse should fill in the information in these columns for **which adherence information is being collected as specified by the protocol.**
- **Column A:** List the drug name (if known or, if double-blinded study, record as marked on bottle).
- **Column B:** List the eight digit drug code for the drug listed in Column A. Refer to Appendix 3 or by using the Drug Code Lookup Program at the DMC Website (www.fstrf.org).
- **Column C:** List the drug color, type (blue pill, pink liquid, etc.) and note any special identifying labels.
- **Column D:** List the expected number of **doses** per 24 hour period. This refers to the **schedule** (e.g. 3 times per day, 4 times per day) and **not** the number of pills. Particulars of the schedule will not be addressed (e.g. TID and q8 hr. would both be recorded as 3 times per day).
- **Columns E-I:** This information is to be obtained from the study subject or primary caregiver in the subsequent interview. Refer to "Scripts for Pediatric Adherence Questionnaire Module 1 – Revised" for completing the Medication Table. This document is located in the Forms Instruction section of the CRF Notebook.

MEDICATION LIST TABLE: Do not key Column C.

[1] Identification Codes	[2] Doses Missed
1-Volunteered without prompt	Enter "-1" if subject isn't sure if he/she missed any doses.
2-Volunteered with prompt	Enter "0" if no doses were missed.
3-Acknowledged when reminded	
4-Did not acknowledge	

Complete Prior to Visit				Complete During Interview				
A	B	C	D	E	F	G	H	I
						Doses Missed[2]		
Drug Name(s) [30]	Drug Code	Drug Color, Type and Labels	Expected # Doses	ID Code[1]	Reported # Doses	Yesterday	2 days ago	3 days ago
1.			☐☐	☐	☐☐	☐☐	☐☐	☐☐
2.			☐☐	☐	☐☐	☐☐	☐☐	☐☐
3.			☐☐	☐	☐☐	☐☐	☐☐	☐☐
4.			☐☐	☐	☐☐	☐☐	☐☐	☐☐
5.			☐☐	☐	☐☐	☐☐	☐☐	☐☐
6.			☐☐	☐	☐☐	☐☐	☐☐	☐☐
7.			☐☐	☐	☐☐	☐☐	☐☐	☐☐
8.			☐☐	☐	☐☐	☐☐	☐☐	☐☐

Language: ☐

Enter an 'E' in the language box if the scripts were read to the subject in English.
Enter an 'S' in the language box if the scripts were read to the subject in Spanish.

12-13-99/03-27-00/07-05-01

Date Form Keyed (DO NOT KEY): _____ /_____ / _____

PEDIATRIC ADHERENCE QUESTIONNAIRE MODULE 2

GENERAL REASONS FOR NON-ADHERENCE

NIAID PEDIATRIC AIDS CLINICAL TRIALS GROUP

Patient Number [ ][ ][ ] [ ][ ][ ][ ] [ ] Date of Patient Visit [ ][ ][ ] [ ][ ] [ ][ ][ ][ ]
mmm dd yyyy

Protocol Number [3][9][0][ ][ ][ ]

Institution Code [ ][ ][ ][ ][ ][ ]

Form Week [ ][ ][ ] * Seq No. [ ] **Step No. [ ][ ] Key Operator Code [ ][ ][ ]

* Enter a "1" if this is the first of this form for this date. Designate subsequent forms on the same date with a 2, 3, etc.
**Enter the subject's current study step number. Enter '1' if the study does not have multiple steps.

This module captures the general reasons for non-adherence linked to each drug taken. In this step, the interviewer will collect information on reasons why the subject may be having difficulty taking each agent. This form should be completed for subjects < 13 years of age. For subjects ≥ 13 years of age, complete the ADOLESCENT ADHERENCE QUESTIONNAIRE MODULE 2 form (QL5020).

QUESTIONS 1 - 3 ARE TO BE COMPLETED BY THE STUDY NURSE:

1. Was the questionnaire completed at this visit? ……..(1-Yes, 2-No) [ ]
   If Yes, go to question 2.
   If No, complete 'a'.

   a. Indicate the reason the questionnaire was not completed:……. [ ]
      1-Subject refused
      2-Primary caregiver refused
      3-Subject missed clinic visit
      4-There was not enough time
      5-Primary caregiver not available
      9-Other reason, specify

   If Other, specify [30]: _____

2. Who responded to the questions?……1-Subject [ ]
   2-Biological Mother
   3-Biological Father
   4-Other Relative, specify
   5-Adoptive parent
   6-Foster parent
   9-Other, specify

   If "4-Other relative" or "9-Other", specify [30]: _____

3. Does the subject know his/her HIV status?…… (1-Yes, 2-No, 3-Information not available/ Not known) [ ]

INSTRUCTIONS FOR COMPLETION OF DRUG SPECIFIC TABLE:

- Enter the name of each antiretroviral drug that the subject is receiving in the space provided at the top of each column.

Identification of Reasons for Non-Adherence:

READ the following paragraph to the subject or primary caregiver:
"Many people at one time or another have trouble with these medications. We would like to better understand the things that make giving medications hard for families. These are some of the reasons others have identified which have made it difficult to take [give] all of the HIV medicines."

Show and read the list of reasons to the subject or primary caregiver.

After the list is read, ask the following question for each drug (question 4 on page 2):
"Over the last two weeks, have any of the following been problems for you with _____(drug name or characteristics)_____?"

If "Yes," enter the frequency code for each reason (a-k).
If "No," go to the next drug.
For data entry, use the tab key after the last entry on the page.

PEDIATRIC ADHERENCE QUESTIONNAIRE MODULE 2
GENERAL REASONS FOR NON-ADHERENCE

Pt. No. ☐☐☐ ☐☐☐☐ ☐ * Seq. No. ☐ ** Step No. ☐☐ Date ☐☐☐ ☐☐ ☐☐☐☐
mmm dd yyyy

DRUG SPECIFIC ADHERENCE DIFFICULTIES:

Frequency Codes

Use these codes to indicate the frequency with which each listed reason for non-adherence occurs. This needs to be done for each antiretroviral drug the subject is taking.

0-Never a problem
1-Hardly ever a problem
2-Frequent problem
3-Almost always a problem

	Drug #1 Name[15]: _____	*Drug #2 Name[15]:* _____	*Drug #3 Name [15]:* _____	*Drug #4 Name[15]:* _____	*Drug #5 Name[15]:* _____
4. Problem identified? (1-Yes, 2-No)	☐	☐	☐	☐	☐
Reasons for Non-adherence:					
a. Can't get drug (drug store doesn't have supply):	☐	☐	☐	☐	☐
b. Didn't refill; ran out:	☐	☐	☐	☐	☐
c. Taste, can't get it down, spits up, amount (pills or liquid):	☐	☐	☐	☐	☐
d. Forgot	☐	☐	☐	☐	☐
e. Side effects/toxicity	☐	☐	☐	☐	☐
f. Scheduling -Interferes with lifestyle (meals, school, sleep)	☐	☐	☐	☐	☐
g. Child refuses	☐	☐	☐	☐	☐
h. Multiple Caretakers	☐	☐	☐	☐	☐
i. Concerns about disclosure	☐	☐	☐	☐	☐
j. Intercurrent illness	☐	☐	☐	☐	☐
k. Other, specify Specify [30]:	☐ _____	☐ _____	☐ _____	☐ _____	☐ _____

Language: English E

09-09-98/09-14-00/07-05-01

Date Form Keyed (DO NOT KEY): _____ /_____ / _____

ADOLESCENT ADHERENCE QUESTIONNAIRE MODULE 2

GENERAL REASONS FOR NON-ADHERENCE

NIAID PEDIATRIC AIDS CLINICAL TRIALS GROUP

Patient Number		Date of Patient Visit	mmm dd yyyy
Protocol Number	3 9 0	Institution Code	
Form Week	*Seq No.	**Step No.	Key Operator Code

* Enter a "1" if this is the first of this form for this date. Designate subsequent forms on the same date with a 2, 3, etc.
**Enter the subject's current study step number. Enter '1' if the study does not have multiple steps.

This module captures the general reasons for non-adherence linked to each drug taken. In this step, the interviewer will collect information on reasons why the subject may be having difficulty taking each agent.

- This form should be completed for subjects ≥ 13 years of age. For subjects < 13 years of age, complete the PEDIATRIC ADHERENCE QUESTIONNAIRE MODULE 2 form (QL5001).
- If the subject has assumed sole responsibility for his/her own drug regimen, the subject should complete this form.
- If the primary caregiver is solely responsible for distributing the medications to the subject, the primary caregiver should complete this form.
- If the responsibility is shared, the subject and the caregiver should jointly complete this form.

QUESTIONS 1 - 5 ARE TO BE COMPLETED BY THE STUDY NURSE:

1. Was the questionnaire completed at this visit? …….. (1-Yes, 2-No) ☐
   If Yes, go to question 2.
   If No, complete 'a'.

   a. Indicate the reason the questionnaire was not completed: …. ☐
      1-Subject refused
      2-Primary caregiver refused
      3-Subject missed clinic visit
      4-There was not enough time
      5-Primary caregiver not available
      9-Other reason, specify

      If Other, specify [30]: _____

2. Does the subject know his/her HIV status? …… (1-Yes, 2-No, 3-Information not available/not known) ☐

3. Was the subject perinatally infected? …….. (1-Yes, 2-No, 3-Information not available/not known) ☐

4. Who is responsible for administering the medications? …… ☐
   1-Primary caregiver solely responsible
   2-Subject solely responsible
   3-Subject and caregiver jointly
   4-Subject and other individual
   9-Other, specify

   If "9-Other", specify [30]: _____

5. Who responded to the questions?…… ☐
   1-Primary caregiver
   2-Caregiver and subject jointly
   3-Subject alone
   9-Other, specify

   If "9-Other", specify [30]: _____

GENERAL REASONS FOR NON-ADHERENCE

Pt. No. ☐☐☐ ☐☐☐☐ ☐ * Seq. No. ☐ ** Step No. ☐☐ Date ☐☐☐ ☐☐ ☐☐☐☐
mmm dd yyyy

INSTRUCTIONS FOR COMPLETION OF DRUG SPECIFIC TABLE:

- Enter the drug code name of each antiretroviral drug that the subject is receiving in the space provided at the top of each column. Refer to the bottom of this page for the list of most commonly used anti-HIV medications. Refer to Appendix 3 or the Drug Code Lookup Program at the DMC Web Site (http://www.fstrf.org) for any medications not listed.

Identification of Reasons for Non-Adherence:

READ the following paragraph to the subject or primary caregiver:
"Many people at one time or another have trouble with these medications. We would like to better understand the things that make giving medications hard for families. These are some of the reasons others have identified which have made it difficult to take [give] all of the HIV medicines."

Show and read the list of reasons to the subject or primary caregiver.

After the list is read, ask the following question for each drug (question 5 on pages 2 and 3):
"Over the last two weeks, have any of the following been problems for you with _____(drug name or characteristics)_____?"

If "Yes," enter the frequency code for each reason (a-r).

If "No," go to the next drug.

For data entry, use the tab key after the last entry on the page.

DRUG CODE REFERENCE

Drug Codes and Names for Commonly Used Drugs.
Refer to Appendix 3 or the Drug Code Lookup Program at the DMC Web Site (http://www.fstrf.org) for drugs not listed below.

Anti-HIV Drugs			
08180407	Abacavir/ABC/Ziagen/1592U89	10920013	Interleukin-2/IL-2
08180025	Alovudine/CL-184824	08180026	Lamivudine/3TC/Epivir
08181205	Amprenavir/APV/Agenerase/141W94/VX-479	08181208	Lopinavir/Ritonavir (LPV/RTV)/Kaletra ABT-378/r
08181214	Atazanavir/ATV/BMS-232632		
08180018	Atevirdine mesylate U-87201E	08180048	Loviride/Lotrene
08180006	Azidouridine/AzdU/azido-2',3'-dideoxyuridine	08181204	Nelfinavir/NFV/Viracept
08180021	AZT/ZDV/Zidovudine/Retrovir	08180013	Nevirapine/NVP/Viramune
08180032	CD4/RST4	08181203	Ritonavir/RTV/Norvir
08180412	Combivir (3TC/ZDV)	08181209	Saquinavir soft gel/FTV/Fortovase
08180024	d4T/Stavudine/Zerit	08180030	Saquinavir (HGC)/SQV/Invirase/R031-8959
08180020	ddC/Zalcitabine/HIVID	08188804	T-20/pentafuside
08180007	ddI/Didanosine/Videx	08182002	TDF/Tenofovir/Tenofovir disoproxil fumarate/Viread
08180031	DLV/delavirdine mesylate/Rescriptor		
08180804	Efavirenz/EFV/Sustiva/DMP266	08180418	Trizivir (3TC/ABC/ZDV)
08180411	Fluorouridine/935U83		
08180415	FTC/coviracil/emtricitabine	99999998	Blinded Study Drug
08181218	GW433908	99999999	Drug Code Pending
08180043	Indinavir/IDV/Crixivan		

06-14-00/08-14-00/07-05-01

ADOLESCENT ADHERENCE QUESTIONNAIRE MODULE 2
GENERAL REASONS FOR NON-ADHERENCE

Pt. No. ☐☐☐ ☐☐☐☐ ☐ * Seq. No. ☐ ** Step No. ☐☐ Date ☐☐☐ ☐☐ ☐☐☐☐
mmm dd yyyy

DRUG SPECIFIC ADHERENCE DIFFICULTIES:

Frequency Codes

Use these codes to indicate the frequency with which each listed reason for non-adherence occurs. This needs to be done for each antiretroviral drug the subject is taking.

0-Never a problem
1-Hardly ever a problem
2-Frequent problem
3-Almost always a problem

Enter Drug Code and Name:	*Drug Code [8]:* _____ *Drug #1 Name [15]:* _____	*Drug Code [8]:* _____ *Drug #2 Name [15]:* _____	*Drug Code [8]:* _____ *Drug #3 Name [15]:* _____	*Drug Code [8]:* _____ *Drug #4 Name [15]:* _____	*Drug Code [8]:* _____ *Drug #5 Name [15]:* _____
6. Problem identified? (1-Yes, 2-No)	☐	☐	☐	☐	☐
Reasons for Non-adherence:					
a. Can't get drug at drug store	☐	☐	☐	☐	☐
b. Didn't get prescription refilled; ran out	☐	☐	☐	☐	☐
c. Made me sick to my stomach; threw up; it tasted bad	☐	☐	☐	☐	☐
d. Forgot	☐	☐	☐	☐	☐
e. It caused me to have other physical symptoms (e.g., rash, headache)	☐	☐	☐	☐	☐
f. Got in the way of daily schedule (school, work); too busy	☐	☐	☐	☐	☐
g. Couldn't deal with it; didn't feel like taking it; needed a break	☐	☐	☐	☐	☐
h. Change in living situation; moved	☐	☐	☐	☐	☐

06-14-00/08-14-00/07-05-01

ADOLESCENT ADHERENCE QUESTIONNAIRE MODULE 2
GENERAL REASONS FOR NON-ADHERENCE

Pt. No. ☐☐☐ ☐☐☐☐ ☐ * Seq. No. ☐ ** Step No. ☐☐ Date ☐☐☐ ☐☐ ☐☐☐☐
mmm dd yyyy

	Drug #1	*Drug #2*	*Drug #3*	*Drug #4*	*Drug #5*
Reasons for Non-adherence (cont'd):					
i. Worried people would find out about HIV; didn't want friends asking questions; felt embarrassed	☐	☐	☐	☐	☐
j. Got sick with another illness; wasn't feeling well (e.g., cold, flu, stomach bug)	☐	☐	☐	☐	☐
k. Don't think I need it anymore; I can stay healthy without it	☐	☐	☐	☐	☐
l. Family and/or friends don't help me remember; tell me I shouldn't take it	☐	☐	☐	☐	☐
m. Nowhere to keep it at school or work	☐	☐	☐	☐	☐
n. Don't understand why I have to take it	☐	☐	☐	☐	☐
o. I keep getting sick even when I do take it	☐	☐	☐	☐	☐
p. Taking it reminds me of the HIV; just want to forget about the diagnosis	☐	☐	☐	☐	☐
q. I don't want to talk about it	☐	☐	☐	☐	☐
r. Other, specify Specify [30]:	☐ _____	☐ _____	☐ _____	☐ _____	☐ _____

Language: English ☐ E

06-14-00/08-14-00/07-05-01

Date Form Keyed (DO NOT KEY): _____ /_____ / _____

APPENDIX 3: ADHERENCE QUESTIONNAIRES USED AT PENTA SITES

4 December 2003

PENPACT 1 PENTA 9 PENPACT 1 PENTA 9 PENPACT 1 PENTA 9

PENPACT 1 STUDY ADHERENCE QUESTIONAIRE

To the doctor or nurse: please complete the following table and write the names, colour and type (eg blue pill, pink liquid) of drugs that the child has been taking in the tables in question 3 and 8.

Clinic Number:	Date of Birth:	**Penpact 1** Trial Number:
Initials:	Date of Assessment:	

Week no. (please ring): 24, 48, 72, 96, 120, 144, 168, 192 Initial regimen ☐ Second regimen ☐	Completed by carer alone? *Yes* ☐ *No* ☐ If **no**, who else was involved? _____	If not completed: Not enough time ☐ Refusal ☐ DNA ☐ Parent/carer not available ☐ Other ☐ *specify: -* _____

To the carer:
We know that it can be difficult giving antiretroviral medicines to children everyday. We are interested in finding out what it is like for you and your family. Please tick the answer which best describes your true situation or feeling, as your answers may help others in the future. Thank you.

1) **What is your relationship to the child ?** Mother ☐ Father ☐
Other (please specify) _____

2) **Who else gives antiretroviral medicines to your child ?** _____

3) **At what time do you usually give your child their antiretroviral medicines?**

Name of drug	Colour, Type	Time of 1st dose	Time of 2nd dose (if 2nd dose given)
1.		**1st dose**	**2nd dose**
2.		**1st dose**	**2nd dose**
3.		**1st dose**	**2nd dose**
4.		**1st dose**	**2nd dose**

4) **Which dose, if any, is the most difficult for you or your child?**
none ☐ morning ☐ lunchtime/after school ☐ evening ☐ all ☐

5) **How easily do you remember to give all the medicines to your child ?**
easily ☐ quite easily ☐ with some difficulty ☐ with great difficulty ☐

6) **What do you use to help you remember to give the medicine?** (tick all that apply)
Labels ☐ Calendar ☐ Pill box ☐ Beeper ☐
MEM Caps ☐ Timer ☐ Programmable wrist watch ☐
Diary ☐ Daily events (eg breakfast time) ☐
Other ☐ *Specify*_____

PENPACT1 PENTA9 PENPACT1 PENTA9 PENPACT1 PENTA9

7) **How much does giving medicines to your child interfere with you/your child's everyday life?**

a lot ☐ quite a lot ☐ not much ☐ not at all ☐

How? _____

_____

8) **How important do you think it is to take the medicines in the way indicated by your doctor (e.g. remembering to take every dose?)**

Extremely ☐ Very ☐ Don't know ☐ Not very ☐ Not at all ☐

9) **Over the last 3 days, can you say how many times, your child has missed a dose:**

No drugs missed ☐

Drug	Yesterday	Day before yesterday	3 days ago
1.	..... dose(s) missed	..... dose(s) missed	..... dose(s) missed
2.	..... dose(s) missed	..... dose(s) missed	..... dose(s) missed
3.	..... dose(s) missed	..... dose(s) missed	..... dose(s) missed
4.	..... dose(s) missed	..... dose(s) missed	..... dose(s) missed

10) **If your child has missed any doses during the last two weeks, please indicate the reason(s) and say which drug(s) :** No drugs missed ☐

Because :		Drug (s)
You had run out of drug(s) ?	☐	
Your child has problems taking some of the drugs?	☐	
You had forgotten?	☐	
You think these drugs are toxic or harmful?	☐	
Taking the drugs is difficult with school hours, meals, sleep etc	☐	
Your child refused to take them	☐	
Your child was being looked after by someone else?	☐	
You did not want other people to know your child was taking these drugs?	☐	
Your child was unwell?	☐	
Your routine, or your child's routine, was different from normal (eg holidays, weekends etc)?	☐	
You were too depressed or unwell?	☐	
You are fed up giving the drugs?	☐	

Further details or any other reason(please specify) :_____

_____

11) Does your child know his or her infection status? *Yes* ☐ *No* ☐

Thank you for taking the time to fill out this form, please add any comments you have:

_____

4 December 2003

PENPACT1 PENTA9 PENPACT1 PENTA9 PENPACT1 PENTA9

PENPACT 1 STUDY
ADHERENCE QUESTIONAIRE FOR PATIENTS

To the doctor or nurse: please complete the following table and write the names, colour and type (eg blue pill, pink liquid) of drugs that the patient has been taking in the tables in question 1 and 8.

To be completed by the doctor or nurse:

Clinic Number:	Date of Birth:	**Penpact 1** Trial Number:
Initials:	Date of Assessment:	
Week no. (please ring): 24, 48, 72, 96, 120, 144, 168, 192 Initial regimen ☐ Second regimen ☐	Completed by patient alone? *Yes* ☐ *No* ☐ If **no**, who else was involved? _____	If not completed: DNA ☐ Not enough time ☐ Refusal ☐ Other ☐ *specify: -* _____

To be completed by the patient

We know that it can be difficult taking medicines everyday. We are interested in finding out what it is like for you and your family. Please tick the answer which best describes your true situation or feeling, as your answers may help others in the future. Thank you.

1) At what time do you usually take your medicines?

Name of drug	Colour, Type	Time of 1st dose	Time of 2nd dose (if 2nd dose taken)
1.		**1st dose**	**2nd dose**
2.		**1st dose**	**2nd dose**
3.		**1st dose**	**2nd dose**
4.		**1st dose**	**2nd dose**

2) Which dose, if any, is the most difficult?
none ☐ morning ☐ lunchtime/after school ☐ evening ☐ all ☐

3) Does any one remind you when to take your drugs? Yes ☐ No ☐

4) How easily do you remember to take all your medicines ?
easily ☐ quite easily ☐ with some difficulty ☐ with great difficulty ☐

5) What do you use to help you remember to take the medicine? (tick all that apply)
Labels ☐ Calendar ☐ Pill box ☐ Beeper ☐
MEM Caps ☐ Timer ☐ Programmable wrist watch ☐
Diary ☐ Daily events (eg breakfast time) ☐
Other ☐ *Specify*_____

PENPACT1 PENTA9 PENPACT1 PENTA9 PENPACT1 PENTA9

6) **How much does taking medicines interfere with your life?**

a lot ☐ *quite a lot* ☐ *not much* ☐ *not at all* ☐

How? _____

_____

7) **How important do you think it is to take the medicines in the way your doctor told you (e.g. remembering to take every dose?)**

Extremely ☐ *Very* ☐ *Don't know* ☐ *Not very* ☐ *Not at all* ☐

8) **Over the last 3 days, can you say how many times, you have missed a dose:**

No drugs missed ☐

Drug	Yesterday	Day before yesterday	3 days ago
1.	..... dose(s) missed	..... dose(s) missed	..... dose(s) missed
2.	..... dose(s) missed	..... dose(s) missed	..... dose(s) missed
3.	..... dose(s) missed	..... dose(s) missed	..... dose(s) missed
4.	..... dose(s) missed	..... dose(s) missed	..... dose(s) missed

9) **If you have missed any doses during the last two weeks, please tick the reason(s) why and say which drug(s):** **No drugs missed** ☐

Because :		Drug (s)
You had run out of drug(s) ?	☐	
You had forgotten?	☐	
You think these drugs are toxic or harmful?	☐	
Taking the drugs is difficult with school hours, meals, sleep etc	☐	
You didn't want to take them	☐	
You did not want other people to know you were taking these drugs?	☐	
You were unwell?	☐	
Your routine was different from normal (eg holidays, weekends etc)?	☐	
You are fed up taking the drugs?	☐	

Further details or any other reason (please specify) _____

_____

_____

Thank you for taking the time to fill out this form, please add any comments you have:

_____

APPENDIX 4: SUPPLEMENTAL FIGURES

Figure S.1. HIV viral load over the first 24 weeks after randomization by adherence on a LOESS plot.

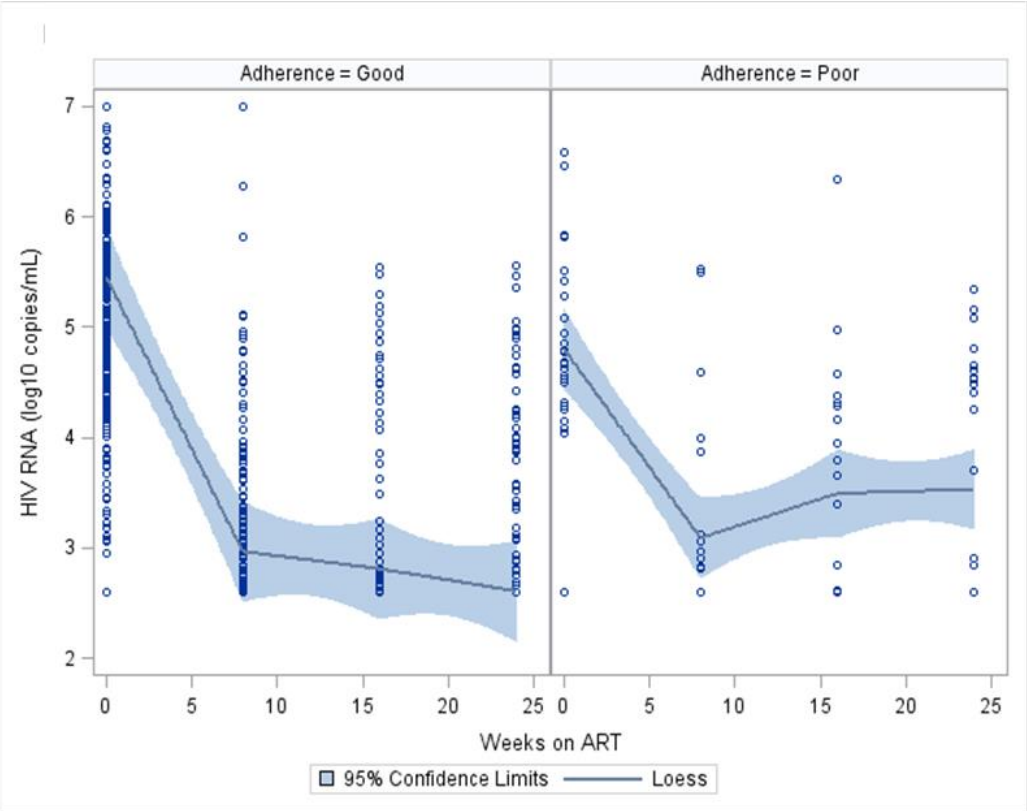
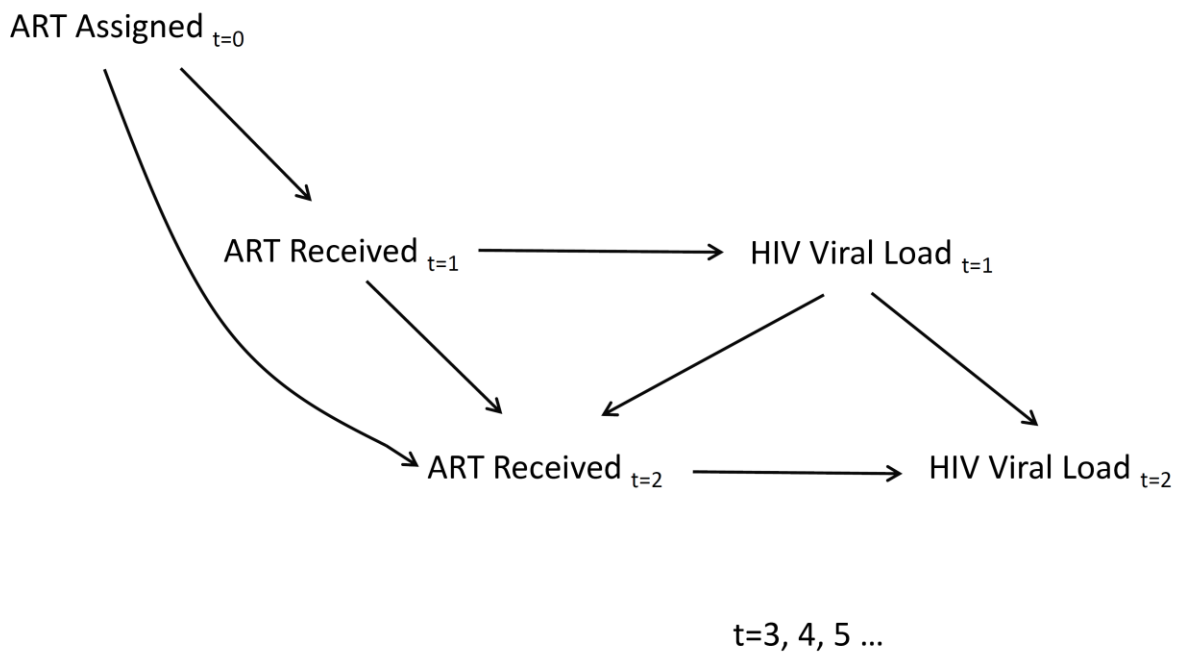


Figure S.2. Causal diagram conceptualizing the relationship among antiretroviral therapy (ART) regimen assigned, adherence, and HIV viral load at multiple time points. This diagram is simplified to illustrate relationships. Adherence to ART is a category of ART received. Protocol non-adherence is defined as a discordance between ART assigned and ART received. t indexes time on study.



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