THE EFFECT OF ANTIRETROVIRAL THERAPY ON PRETERM BIRTH IN A U.S POPULATION OF WOMEN LIVING WITH HIV: A REEXAMINATION OF ANALYSIS METHODS

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ABSTRACT

Hamsa Lakshmi Subramaniam: The Effect of Antiretroviral Therapy on Preterm Birth in a U.S Population of Women Living With HIV: A Reexamination of Analysis Methods
(Under the direction of Audrey Pettifor)

Harmful effects of combination antiretroviral therapy (cART) in pregnancy is a research priority given its widespread use in preventing vertical transmission of HIV. Studies investigating the relationship between ART and preterm birth (PTB) offer conflicting results as to whether PI cART is harmful in causing PTB, likely due to methodological challenges and not truly harmful effects. Our study reexamines methods used in the literature with the Women and Infants Transmission Study data (WITS). WITS is a US-based observational cohort designed to study the natural course of maternal-infant HIV-1 infection between 1990 and 2005. Exposure categories considered for both aims are no therapy, Zidovudine (ZDV) monotherapy; PI-based cART and non-nucleoside reverse transcriptase inhibitor (NNRTI) cART. For Aim 1, we used the complement of the Kaplan-Meier estimator and inverse probability weights to estimate PTB risk by time-varying exposure. A total of 1,067 HIV-positive pregnancies in 932 women were followed until delivery. The weighted risk differences indicated PI cART was harmful compared to NNRTI cART in preventing PTB, though insignificant. Exposure to either combination therapy were significantly associated in preventing very preterm compared to other exposures. For Aim 2, we demonstrated target trial emulation to examine the intention-to-treat effect of exposure on PTB. Women were enrolled and assigned treatment between 18 and 22 weeks to emulate trial enrollment at 20 weeks. We assumed that exposure assignment was conditional on measured baseline covariates to emulate baseline randomization. Log-Poisson models with

robust variance estimators were used to report risk and risk ratios with 95% confidence intervals. Two hundred and six women were assigned their enrollment exposure. After adjusting for baseline covariates, women starting PI cART at 20 weeks had increased risk of PTB when compared to all other exposures, though all effect estimates were statistically insignificant. This finding is contrary to what is established in the ART literature. Although the WITS was not the ideal candidate for demonstrating the use of survival analysis or target trial emulation, both methods can accommodate the realities of observational cohort data and should be considered as an alternative to conventional binary methods.

To my children,
who are a constant reminder of what matters most

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LIST OF ABBREVIATIONS

3TC	lamivudine
ART	antiretroviral therapy
AZT	azidothymidine
cART	combination ART
BMI	body mass index
CD4	cluster of differentiation 4
CDC	Centers for Disease Control
CI	confidence interval
DAG	directed acyclic graph
ELISA	enzyme-linked immunosorbent assay
EMM	effect measure modification
FDA	Federal Drug Administration
FDC	fixed-dose Combinations
GA	gestational age
GEE	generalized estimating equations
HAART	highly active antiretroviral therapy
HIV	human immunodeficiency virus
INSTI	integrase strand transfer inhibitor
IPW	inverse probability weights
IQR	interquartile range
LMP	last menstrual period
LPV	lopinovir
MI	multiple imputation
NIAID	National Institute of Allergy and Infectious Diseases

NNRTI	non-nucleoside reverse transcriptase inhibitors
NRTI	nucleoside reverse transcriptase inhibitor
NVP	nevirapine
PI	protease inhibitors
PTB	preterm birth
RCT	randomized control trial
RD	risk differences
RR	risk ratios
RNA	ribonucleic acid
RTV	Ritonovir
WITS	Women and Infants Transmission Study
ZDV	Zidovudine

CHAPTER 1: SPECIFIC AIMS

An increasing number of HIV-infected women of reproductive age are initiating antiretroviral therapy worldwide. Because of this, the number of women on treatment before and during pregnancy is increasing. Modern HIV treatments, known as highly active antiretroviral therapy (HAART), effectively prevent vertical transmission from mother to child, contributing to a growing population of individuals living without HIV, due to in-utero exposure to antiretroviral drugs. Current research priorities have turned to the effect of treatment initiation timing and length of exposure to certain classes and combinations of antiretroviral drugs that could be associated with preterm birth and other adverse birth outcomes. This study analyzed data from the Women's Infant Transmission Study (WITS), to further refine our understanding of the possible effect of HIV treatment type on preterm birth (delivery prior to 37 weeks gestation) using revised methodological approaches than those used in the literature.

The WITS cohort is a large, multi-site study of HIV-positive women in pregnancy and their infants collected in the United States from 1988 to 2005, with follow-up ending in 2008. While related questions have been explored using these data in the past, this study proposes a re-analysis of the WITS data for the following reasons:

1. The published analysis of preterm birth outcomes includes women in the cohort enrolled between 1990 and 2002, however enrollment continued until 2005.[3] The proposed analysis would consider an additional 600 women enrolled between 2002 and 2005.

2. Analyses examining the relationship between preterm birth and HIV treatment are subject to a range of methodological flaws. Perhaps the most common of these are erroneously

adjusting for prior preterm birth and inappropriately utilizing binary methods in the context of longitudinally collected observational data. Binary regression methods do not easily accommodate time-varying exposure, competing risks or censoring, which are common features of pregnancy cohorts. Reanalyzing to estimate preterm birth risk utilizing survival methods or appropriately using binary methods to estimate easily interpretable measures of effect would be more appropriate given the presence of late-entry and time-varying exposure to HIV treatment as well as to facilitate public health action.

To explore the use of alternative epidemiology methods to understand the effect of in-utero exposure to PI-based ART on preterm birth, this dissertation has two aims.

Aim 1: Estimate the effect of ART regimen on risk of preterm birth among HIV-infected pregnant women enrolling after 1996 who initiate treatment after conception and prior to 28 weeks gestation using survival methods. *We hypothesize that the effect of PI ART on preterm birth will not be different than that among pregnancies exposed to other combination therapies.*

Aim 2: Estimate the causal effect of ART regimen initiated at 20 weeks gestation on risk of preterm birth among HIV-infected pregnant women by emulating a target trial using observational data. *We hypothesize that the effect of PI ART on preterm birth derived from the emulated target trial will not be different than that among pregnancies exposed to other combination therapies, but will be protective compared to monotherapy and no therapy.*

CHAPTER 2: BACKGROUND

2.1 HIV and Pregnancy in the United States

The public health burden of HIV remains high worldwide, particularly among reproductive-aged women of color. Of the 34.5 million adults living with HIV globally in 2016, nearly half of the population were women between 16 and 45 years old.[4] In that same year, adult and adolescent women comprised approximately 20% of all new HIV diagnoses.[5,6] In the United States, HIV incidence among adults has declined overall since treatment and prevention efforts gained momentum starting in the early 1990s. Even so, HIV incidence among Black women of reproductive age is 20 times higher compared to white women - a difference that has not meaningfully changed from the mid-1980s when HIV was first discovered among women in the United States.[6–8] As of 2017, 69% of new HIV cases were Black or Latino, and 19% of new cases were women of reproductive age.[9] As a result, women of color are more likely than all other racial groups combined to conceive and give birth while living with HIV.

Pregnant women are a special population within those living with HIV due to the additional risk of transmitting HIV to their infant. The estimated number of women living with HIV who give birth in the United States has increased by 30% between 2000 and 2017.[10] Transmission of HIV to an infant is most likely to occur during the intrapartum and post-partum periods. During the intrapartum period marked by labor and delivery, significant mixing of maternal and fetal blood and fluids occurs, exposing the fetus to infection. Post-partum transmission of HIV occurs when a woman passes HIV to her child via breastmilk containing the virus.[11] Without any ART, an estimated 15-30% of babies born to HIV-positive mothers will

acquire HIV in-utero[12,13] or in the intrapartum period, and an additional 5-40% will be infected through breastfeeding. [11,14,15]

Health outcomes for pregnant women living with HIV and their children have improved with the introduction of new drugs and interventions since the early 1990s. HIV-testing for pregnant women; HIV treatment provision during pregnancy, delivery and post-partum periods; avoidance of breastfeeding, and elective cesarean section dramatically reduced rates of vertical transmission in the United States from 43.1 per 100,000 births in 1992 to 1.8 per 100,000 births in 2013.[16,17] Until 2000, women and clinicians generally decided on an ad hoc basis whether C-section was appropriate given the overall pregnancy context. If a woman was severely infected with HIV, indicated by a high viral load in her blood, a C-section delivery would avoid the mixing of infected maternal fetal blood during spontaneous vaginal labor, thereby minimizing infant risk of exposure to HIV.[18] Similarly, early guidelines advised exclusive formula-feeding to prevent infant exposure to infected breastmilk altogether. Since 2012 however, advances in HIV treatment type, availability and adherence, coupled with improved obstetric care have allowed HIV-positive pregnant women to experience pregnancy, delivery, breastfeeding and post-partum periods normally provided they are able to achieve viral suppression.[4,19]

2.2 HIV Treatment in Pregnancy

The most significant of these interventions in preventing vertical HIV transmission widespread HIV testing and initiating women on ART during pregnancy. The first Centers for Disease Control (CDC) recommendations on preventing mother-to-child transmission in 1987 recommended that women delay pregnancy and avoid breastfeeding until more is known about the risks of transmission.[20] The first HIV antiretroviral therapy discovered was a nucleoside reverse transcriptase inhibitor (NRTI) called azidothymidine (AZT), approved by the Federal Drug Administration (FDA) 1987. AZT was later renamed zidovudine (ZDV) and was shown to

be effective for 98% of non-pregnant adults through 24 weeks of treatment, however the beneficial effects declined shortly after.[21] Even so, the United States Public Health Service recommended antenatal oral administration of ZDV for pregnant women, beginning at 14-34 weeks and continuing throughout pregnancy to prevent vertical transmission.[18] It was further recommended that women receive intrapartum intravenous ZDV to suppress viral load during delivery, followed by postnatal oral administration of ZDV to the infant for six weeks after delivery.[22] The use of this three-part regimen of ZDV monotherapy rapidly became standard practice. Consequently, vertical transmission risk was reduced by 67.5% in women with CD4 cell counts > 200 cells/mm.[22,23]

Additional NRTIs were developed and put on the market, however all of them faced similar challenges as ZDV when administered alone.[24] When HIV's highly mutagenic properties were discovered, treatment with one drug caused resistance and eventual inefficacy of treatment. Given the limitations of ZDV monotherapy, the research and clinical communities hypothesized about the possible benefits of treating HIV with more than one drug simultaneously, however more drug development was necessary to test this hypothesis. After the introduction of ZDV in 1987, the next drug to market in 1995 was Saquinavir (SQV), a PI.[24] Shortly thereafter, Nevirapine (NVP), the first of the non-nucleoside reverse transcriptase inhibitors (NNRTI), was approved.[24] HIV medications and the year of FDA approval are detailed in Table 2.1.

The discovery of combination therapies known as HAART (referred to as combination ART, cART in subsequent chapters) in 1996 led to substantially better survival and immune recovery than prior treatment regimens. Despite the development of new classes of drugs, mono- and dual- therapy still did not offer sustained viral suppression and immune recovery.[24,25] Trials confirmed that combinations with three drugs led to durable viral suppression and continues to

be the standard of care in 2020.[26] After the discovery of HAART in 1996, HIV-positive adults meeting the recommended CD4 thresholds were advised to initiate or switch to HAART (Table 2.2). Pregnancy treatment guidelines extended from the adult guidelines with modifications, usually based on possible side-effects for the woman or toxicities for the child.[27] A considerable amount of evidence demonstrated that early initiation to treatment increased likelihood of morbidity-free survival and reduction in HIV transmission. In March of 2012, HIV guidelines recommended universal "test and treat", removing all CD4 eligibility thresholds and encouraging clinicians to initiate treatment upon a HIV diagnosis.[4,28] As a result, perinatal transmission rates in the United States dropped from 42.8 per 100,000 live births in 1991, to 1.3 per 100,000 live births in 2017. [29,30]

With the science of mother-to-child HIV prevention now largely established and the worldwide incidence of pediatric HIV on the decline, attention has turned to adverse effects of type and timing ART exposure in pregnancy.[31] Preterm birth is a primary cause for a host of short and long-term developmental challenges faced by children.[32] If there is indeed a significant association between ART and preterm birth, exploring alternative HIV treatment regimens may prevent adverse birth outcomes for the 270,000 HIV-positive women of reproductive age and their children in the United States alone.[9]

2.3 ART and Preterm Birth

The etiology of preterm birth is the principle unsolved problem in perinatal medicine. Preterm birth accounts for 75% of perinatal mortality worldwide, and more than half of long-term morbidity of children, including cognitive disability, poor motors skills, behavioral problems, hearing loss, and chronic lung disease.[33–36] HIV is known to be associated with preterm birth. Prior to the scale-up of HIV treatment in the United States, approximately 35% of HIV-exposed babies were born preterm, compared to 10% among HIV-negative women. [36,37]

Women living with HIV share other risk factors for preterm birth, like gestational diabetes and gestational hypertension; [3,38] excessive alcohol and drug use;[34,36] and smoking cigarettes both prior to and during pregnancy.[37] It is clear that the causes of preterm birth are complex, particularly among HIV-positive women.

A growing literature suggests that certain types of ART could cause preterm birth. In 1998, a Swiss group reported an increased risk of preterm birth among women receiving HAART in pregnancy.[41] Since then, a multitude of studies examining the relationship between ART and adverse birth outcomes have emerged from all over the world, using surveillance, observational and trial data.[35,36,42,43] Risks have been suggested both by regimen type, and timing of HIV initiation as it relates to conception. The protease inhibitor class of drugs has been commonly implicated in causing preterm birth, both in high- and low- income contexts.[44–46] Additionally, preconceptional ART initiation has been suggested to cause preterm birth compared to women initiating ART during pregnancy.[47–50]

The suggestion that preconceptional exposure to HIV treatment causes preterm birth is "at odds with biology."[51] Findings from the PROMISE trial conducted in several sites sub-Saharan Africa report that women who initiated any ART combination preconceptionally were more likely to experience preterm birth compared to women who initiate ART during pregnancy.[47,48] Similar results were found in studies conducted in Botswana[49] and Ireland.[50] Extending these findings, longer duration of ART exposure with initiation prior to pregnancy have shown the highest rates of preterm birth.[48,52,53] Preterm birth occurs as a result of premature initiation of the labor cascade, marked by acute inflammation. Any untreated infection causes immune activation and systemic inflammation. Controlling HIV infection through ART will minimize the inflammatory response, thereby lowering preterm birth risk. Use of ART prior to

conception would therefore reduce, and not increase, the risk of preterm birth.[54,55] Even so, it is possible that certain ART classes – while preventing transmission of HIV - contain inflammatory agents that could cause premature initiation of the labor cascade.[38] If treatment for HIV is in fact causing premature delivery in addition to the infection, further study is of urgent importance considering the risks of lifetime disability and death of the child.

Even so, these conclusions could be subject to systematic selection bias making it seem that risk of preterm birth is higher among preconceptional ART users, when in reality, there is no such risk.[51,56] While HIV treatment exposure later in pregnancy could be associated with iatrogenic preterm birth, a study from Spain found that early exposure is not associated with spontaneous preterm birth at all.[57] Perhaps most convincing is that treating HIV infection as early as possible will always be better than the alternative because sustained HIV infection is known to not only cause HIV transmission, but also to be strongly associated with preterm birth.[54,55] Further comparative evaluations of treatment regimens utilizing appropriate analysis methods are needed.

2.4 Limitations in the Current Research

Studies exploring the effect of ART type and timing of exposure on preterm birth are limited in three primary ways. First, studies can be prone to systematic selection bias. Timing of ART initiation with respect to gestational age is commonly reported as a risk factor for preterm birth. Several observational studies conducted in Europe, the United States, and sub-Saharan Africa have reported that preconceptional ART is associated with increased risk or odds of preterm birth.[43,49,58–61] As mentioned previously, this finding is inconsistent with biological plausibility and is likely due to the systematic exclusion of women who initiate ART late in pregnancy or after delivery.[51,56] Women initiating ART after delivery are often removed from the risk set (even if they experienced preterm birth) due to poorly defined inclusion criteria

conditioning on exposure early in gestation. This makes it appear that those continuing on preconceptional ART have a higher risk of preterm birth in comparison.[51] One way to address this type of bias is to include all fetuses at risk of the outcome – independent of initiation timing – in estimating risk.[62] By extension, cohort membership is defined by current exposure, however observational analyses of extant data often inadvertently violate this principle by using "future exposures" to define present, analytic cohort membership inducing both selection bias and possibly immortal person-time.[63]

Second, inferences can be limited by the use of inappropriate analysis methods. A vast majority of studies examining HIV and preterm birth are prospective or retrospective cohort studies, which utilize logistic regression to estimate odds ratios. Logistic regression assumes that the exposure is consistent across the specified observation period, which is often untrue in these data. In the context of HIV pregnancy, women can initiate treatment at any time during pregnancy, contributing both exposed and unexposed time to the overall gestational period. Women enrolled in cohort studies also usually have unequal observation times based on when she attends her first prenatal visit and could experience a competing event like miscarriage, which could erroneously exclude her from the risk set (depending on how cohort membership is specified). Time-to-event methods are best suited to deal with time-varying exposure, late-entry and competing risks, but few studies employ these methods to explore the effect of ART on preterm birth.

And finally, the effect estimates derived are not always useful to clinical decision-making. Studies regularly report statistical associations instead of endeavoring to make causal claims. Analysis methods which clearly approach making causal claims facilitate public health action and policy. A measure of association commonly reported in the literature is the odds ratio,

produced using logistic regression. Odds ratios tend to be commonly understood as risk ratios, even though odds overestimate the true risk.[64,65] The margin of overestimation increases as the outcome becomes more common. This is problematic in the context of HIV-positive pregnancies, given that preterm birth is a relatively common outcome in this population. This is easily addressed by simply choosing a different model to directly estimate risk. Additionally, estimating risk allows the effect to be presented as a risk difference, such that it is easily calculated and understood as the difference in risk between exposures of interest.[66]

Another way to estimate effects using observational data is to emulate a target trial.[67–70] In the most literal sense, a target trial is a hypothetical RCT that we would wish to conduct under ideal circumstances. Thinking about studies in this way offers a useful heuristic to clarify the study design and the corresponding claims of effect we wish to make.[67] When emulating a target trial with observational data, the target trial is the RCT we would design with the variables contained within the observational dataset we intend on using, assuming that the dataset contains sufficient information on confounders to approximate baseline randomization.[68,69] If the emulation is successful, the results would be comparable to results from the target trial, had it been conducted. The value in analyzing observational data using the target trial lens lies in the ability to approach causal inference explicitly, as opposed to the implicit (and perhaps informal) attempts at causal analyses using observational data.[69]

2.5 Conclusion

Our study is the first to explore the use of alternative epidemiologic analysis methods to promote interpretability and comparison across studies of observational data in the HIV and pregnancy space. It is also the first to explore the use of the emulated target trial approach using an HIV and pregnancy dataset to facilitate causal claims regarding the effect of ART on preterm birth. For Aim 1, we hypothesize that the effect of PI cART on preterm birth will not be different

than that among pregnancies exposed to other combination therapies. The prevailing biological and epidemiological evidence suggests that the benefit of exposure to any combination ART regimen, irrespective of drug class, outweighs the alternatives of not being treated or treatment with monotherapy. Further, the harm conferred by PIs (as suggested by numerous studies) is likely due to methodological flaws in the current literature and not to an inherent pharmacologic quality that initiates premature spontaneous labor onset. For Aim 2, we hypothesize that in the emulated target trial analysis, the effect of PI ART on preterm birth will not be different than that among pregnancies exposed to other combination therapies, but will be protective compared to monotherapy. The emulated target trial uses an intent-to-treat approach by utilizing baseline exposures to estimate the causal risk ratio of the effect of ART on preterm birth.

If ART regimen type is meaningfully associated with preterm birth in the WITS cohort, while utilizing appropriate methods and measures, the findings have the potential to influence the methodological approach of future HIV research. Identifying and demonstrating the use of refined methods to motivate clinical practice and public health action can harmonize methods in the field of HIV and pregnancy. This would allow for comparisons between studies to be conducted more easily which would ultimately clarify evidence for HIV treatment policies and programs. Preventing preterm birth and other adverse birth outcomes associated with ART can have a major public health impact by reducing infant mortality and morbidity, as well as protecting the reproductive goals of women living with HIV, and for their children and families.

2.6 Tables and Figures

Table 2.1. HIV medications by drug class and year of FDA approval from 1987 to 2005 (Drugs no longer in use are listed in italics). [47,71]

Nucleoside reverse transcriptase inhibitors (NRTIs/NtRTI)	**Protease Inhibitors (PIs)**	**Non-nucleoside reverse transcriptase inhibitors (NNRTIs)**	**Fixed-dose Combinations (FDC)**
ZDV – Zidovudine (1987) ddT – Didanosine (1991) 3TC – Lamivudine (1995) ABC – Abacavir (1998) TDF – Tenofovir (2001) FTC - Emtricitabine (2003)	SQV – Saquinovir (1995) RTV – Ritonovir (1996)* LPV – Lopinovir (2000) ATZ – Atazanavir (2003) FPV - Fosamprenavir (2003) DRV – Darunavir (2006)	NVP – Nevirapine (1996) EFV – Efavirenz (1998)	2 PIs - LPV/RTV - Kaletra (2000) 3 NRTIs - ABC/3TC/ZDV – Trizivir (2000) 2 NRTIs - TDF+FTC – Truvada (2004) 1 NNRTI + 2 NRTIs - EFV/FTC/TDF – Atripla (2006)
d4T - Stavudine (1994)	*IDV - Indinivir – (1996)* *NFV - Nelfinavir – (1997)* *APV - Amprenavir – (1999)*	*DLV - Delavirdine (1997)*	

*In 2001, boosting with RTV was recommended for all PIs.

Table 2.2 HIV treatment guidelines in the United States for adults and pregnant women from 1994 to 2012.[4,18,20]

Year	Adults > 18 years	Pregnant Women
1994	Initiate if CD4 < 200/ml	*3-Part ZDV* • Daily oral ZDV (starting 14-34 weeks gestation) • Intravenous ZDV during labor • Oral ZDV for infant 6 weeks postpartum
1996	HAART if CD4 <350/ml	Treatment Naïve: 3-part ZDV Regimen Not Treatment Naïve: switch out NNRTI for ZDV if not already part of combination therapy
2000	HAART if CD4 <350/ml or plasma HIV RNA > 10,000 copies	Treatment Naïve: HAART + 3-part ZDV; can delay initiation until after 12 weeks gestation Not Treatment Naïve: • Switch out NNRTI for ZDV if not already part of combination therapy • Option to stop therapy prior to 12 weeks, and restart after 12 weeks No treatment during gestation: • Single dose NVP at labor onset + single dose NVP to baby *or*; • Oral ZDV and 3TC during labor + one week of oral ZDV/3TC for newborn *or;* • Intravenous ZDV during labor + 6 weeks oral ZDV to newborn *or;* • 2-dose NVP with intravenous ZDV during labor + 6 week oral ZDV to newborn
2004	• Initiate if CD4<350 and >200 • For asymptomatic, treatment naïve patients with CD4+ T cell count >350 cells/mm3, consider initiation if viral load is >55,000 copies	No change
2005	No change	Treatment Naïve: same as adult guidelines, but avoid EFV/NVP Not Treatment Naïve: • After 12 weeks, switch to ZDV-based combination therapy • Women on NVP-based therapies should be monitored • Women on EFV-based therapies should be switched No treatment during gestation: 3-part ZDV
2009	Initiate if CD4<500	No change
2012	ART for all, irrespective of CD4 count	No change

CHAPTER 3: RESEARCH DESIGNS AND METHODS

3.1 Study Overview

Overall, the objective of the study was to reexamine the methods used to understand the relationship between type of antiretroviral therapy on preterm birth among women living with HIV. We approached this objective in two ways using the Women and Infants Transmission Study (WITS). The first aim analyzed the effect of time-varying exposure to ART on preterm birth using survival methods. This is contrast to the analysis performed by the study team using logistic regression.[3] We compared the effect of exposure to combination therapies No Therapy, ZDV monotherapy and NNRTI cART to PI cART initiated in pregnancy on preterm birth among women enrolled from July 31, 1996 to end of enrollment in 2005. The second aim estimated the causal effect of ART regimens on preterm birth among women enrolled in the larger cohort between 1990 and 2005 by emulating a target trial.[67] These analyses answer similar research questions but demonstrate the use of two different analysis approaches to make inferences about ART use in pregnancy.

3.2 Parent Study and Study Population

The Women and Infants Transmission Study (WITS) is a US-based, multi-center, prospective, interval cohort designed to study the natural course of maternal-infant HIV-1 infection. Between July 1989 and December 2005, HIV-positive pregnant women between the ages of 15 and 44, were eligible for enrollment on a continuous basis and at any point in gestation. The entire study population were confirmed to be HIV positive at the enrollment visit. Participants were tested using enzyme-linked immunosorbent assays (ELISA), with subsequent

HIV diagnosis confirmation using Western Blot. Pregnancies were then followed-up at gestational weeks 18±2, 25±2, 34±2 and at delivery, corresponding to visits 1, 2, 3, and 4. Post-partum, both infants and their mothers were followed-up at 4-6 months intervals until study end or they were lost to follow-up. It was possible for the same women to be enrolled more than one time corresponding to multiple pregnancies.

Prospective data collection included structured patient interviews and standardized clinical and laboratory assessments at each study visit. Retrospective data collection consisted of medical record abstraction. Study sites included are hospitals in Boston, MA; Worcester, MA; New York, NY; Houston, TX; San Juan, PR; and Chicago; IL. Informed consent was obtained for all women and infant participants according to each site's local institutional review board, alongside federal guidelines for research. All prenatal care was at the discretion of the clinician. For the purposes of the proposed study aims, this is most important when understanding the circumstances of treatment decision-making (timing, switching and regimen type, etc.), as well as labor and delivery-related decisions (labor induction, methods of assisted delivery, cesarean-section, etc.). The WITS cohort spanned nearly two decades of changing HIV-treatment guidelines, which also affected treatment decisions made by clinicians with their patients over time. Women pregnant after 1996 were likely eligible for some version of HAART (hereafter referred to as cART), marking a period of relatively less variation in the recommended HIV-treatment for pregnant women, and more comparable to treatment options available currently.

3.3 Analytic Sample Construction

For aim 1, we excluded women from the original study who were enrolled prior to 1996 with the intention of analyzing pregnancies exposed to ART regimens relevant to clinical practice. PIs and NNRTIs are still currently prescribed, but given that the WITS study ended in 2005 prior to the introduction of the new drug classes commonly used today (namely Integrase

Inhibitors), our analysis still considers outdated regimens in including ZDV monotherapy. Pregnancies were also excluded if they were exposed to ART at conception; were multiple gestation; ended prior to 20 weeks gestation; and if they enrolled after 28 weeks gestation.

For aim 2, we identified eligible subjects based on articulating the ideal target trial to estimate the causal effect of ART initiated at 20 weeks gestation on preterm birth, and designing our trial emulation sample accordingly. The analysis sample therefore comprised of women enrolled into WITS between 18 and 22 weeks gestation at any time during the study period and whose pregnancies survived to 20 weeks. Further detail on subject typology and exclusion criteria as they relate to pregnancy treatment guidelines are in Table 3.1.

3.4 Gestational Age Assessment

Gestational age (GA) is the underlying measure estimated for each pregnancy at entry, at each study visit, and at delivery. GA and length of pregnancy were calculated according to agreement between last menstrual period (LMP) as reported by the mother and first available ultrasound.[72] In cases with disagreement between these two measures, GA was determined using ultrasound only and/or neonatal physical examination.

3.5 Outcome Assessment

Pregnancies can end at any point after conception. The timing of pregnancy termination is a key component in categorizing the type of birth outcome. Loss of the fetus prior to 20 weeks gestation is generally defined as an *abortion* and can be either spontaneous or elective. Spontaneous loss is commonly referred to as miscarriage. Live births between 20 and 37 weeks is considered preterm. Still births combined with live preterm births will also be considered as preterm in this analysis as a secondary outcome, given the shared causal pathway between ART exposure and delivery. *Extremely preterm* is a subset of preterm birth defined as occurring between 16 weeks 0/7 days and 28 weeks 6/7 days gestation. Births are considered very preterm

if they occur between 28 weeks 0/7 days and 31 weeks 6/7 days gestation; and late preterm describes births occurring between 32 weeks and 36 weeks 6/7 days.

Preterm birth can occur either spontaneously or due to medical intervention, known as iatrogenic birth. Iatrogenic births are initiated by the clinician in the presence of possible risks to the mother or infant, such as preeclampsia, infection, or abnormal fetal testing. Due to the possible differences in causal pathways between births occurring spontaneously and due to medical intervention, reporting the risk of spontaneous preterm birth only could further clarify the effect of PI cART We have therefore identified only those preterm births that could be affected by ART exposure in pregnancy, either occurring by spontaneous onset of labor or medically indicated delivery due to an underlying condition known to be associated with ART. Women carrying more than one fetus have a higher risk for experiencing a range of pregnancy complications that could lead to poor birth and infant outcomes. Since we are not interested in this particular causal pathway for the proposed analysis, multiple gestation pregnancies were excluded for all aims.

3.6 Exposure Assessment

Information pertaining to time of ART use in the 12 months prior to enrollment were collected at enrollment visits and for each study visit based on medical record abstraction. If medical records were unavailable, information on ART use was based on subject recall. Time was collected as month and year and then converted to months since enrollment or months since diagnosis in the dataset. Exact dates of treatment initiation and stopping treatment were not recorded. Time was later transformed into gestational weeks. Exposure to ART is relevant for all three study aims. Exposure definitions for the proposed analysis have two components – timing of exposure and regimen type.

3.7 Timing of Exposure

For both aims, timing of treatment initiation depended mainly on disease severity measured by CD4 count and/or viral load at the time. Table 3.1 describes the types of subjects in WITS based on possible treatment plans CD4 thresholds between 1996 and 2005 and timing of initiation. If a woman living with HIV was diagnosed prior to pregnancy, she could have initiated treatment if she met the disease severity thresholds, being preconceptionally exposed to ART. In contrast, women who were diagnosed with HIV but did not meet the disease severity threshold at the time of diagnosis, or those who were diagnosed in pregnancy were likely to have not been initiated irrespective of CD4 count or viral load. Pregnant women living with HIV could be initiated at any time during pregnancy at the discretion of her clinician. Only women starting HIV treatment in pregnancy were analyzed in this study.

While the exact date of initiation and date of conception are unknown in these data, we assumed that that women initiating HIV treatment prior to enrollment in WITS were on treatment while conceiving. Women who were exposed to ART in pregnancy without evidence of having been exposed prior to enrollment were assumed to have been unexposed to ART at conception and were included in the analysis for both aims (Types 3, 4 and 5 in Table 3.1). Table 3.1 describes the possible scenarios of treatment initiation, given the CD4 thresholds for initiation at the time, the data that are available, and the assumptions we made to categorize initiation time. Exposure was considered time-varying for Aim 1, and baseline exposure was carried throughout gestation to delivery, approaching an intention-to-treat analysis for Aim 2.

3.8 Regimen Types

The proposed analysis focuses on the effect of PI-based ART compared to other exposures pregnant women had during the study period. A significant proportion of women in the parent study did not receive any therapy until later in pregnancy or at delivery. This is

consistent with the introduction of novel ART regimens in the mid-1990s. For Aim 1, we defined exposure using data collected at each study visit, based on both patient recall and medical record abstraction. Each study drug was entered into the database with a start date and stop date. These times were converted into "time since enrollment" values. We then reconstructed multi-drug, combination regimens using the start and stop dates to define our study exposures. If a pregnancy were exposed to a regimen for less than two weeks, it was discarded and the regimens on either side of this <2 week window split the difference. Because the purpose of WITS was to study the natural course of HIV in pregnancy in the context of changing therapy guidelines, we thought it safe to assume that no drug data meant that the woman was unexposed to treatment; not that treatment data were missing.

Therefore, pregnancies exposed to at least one PI with at least two NRTIs were categorized as PI cART exposure; at least one NNRTI with at least two NRTIs was categorized as NNRTI cART. ZDV exposure without other accompanying drugs or reported concurrently with either PI- /NNRTI- cART was categorized as monotherapy; and gaps in treatment were considered as exposure to no therapy. ZDV monotherapy was the standard of care during pregnancy prior to the discovery of combination regimens. These categorizations are further detailed in Appendix B. After 1996, ZDV monotherapy was given concurrently to cART regimens. For both aims, if a pregnancy was exposed to both a combination regimen and ZDV monotherapy, the combination regimen was prioritized for categorization. For the Aim 2 analysis emulating a target trial, we considered baseline exposure only. This meant that among women who were eligible and unexposed to treatment at conception, women were separated into exposure groups based on their treatment regimen at enrollment, occurring among women enrolling between 18 and 22 weeks gestation. The exposure groups considered for the emulated

trial were identical to that defined for Aim 1 – No Therapy, PI cART, ZDV monotherapy and NNRTI cART.

3.9 Covariate Assessment

Covariates detailed in Table 3.2 were measured during study follow up and will be considered in estimating effects in the proposed analyses. Because this dataset is publicly available, certain variables have been recategorized or omitted to protect subject privacy, especially since so few infants were born with HIV in the later years of the study. Therefore, study site has been removed entirely from the dataset, and exact year of birth has been categorized as "1996+" for all births occurring 1996 and onward. Other potentially relevant variables, such as maternal educational attainment and insurance status were collected but not included in the cleaned WITS analysis datasets. Additional covariates relevant to specific aims are described in the corresponding sections below. The causal pathway for each aim is described using a causal diagram represented as a directed acyclic graph (DAG) in Appendix A. Covariates determined to be part of the minimally sufficient adjustment set were included in the final effect estimates.

- *Functional Form Assessment of continuous variables:* We used restricted quadratic splines with four equally placed knots for all continuous variables for the final adjusted models.
- *Effect Measure Modification (EMM):* EMM will not be considered for either analysis aim.
- *Missingness:* We assessed whether missing values of covariates are conditional on either the exposure or outcome, and subsequently whether it introduces bias to the effect estimate. If baseline values were assumed to be missing at random, multiple imputation

or inverse probability weights were used to account for these missing data, depending on the analysis model used for the primary analysis. If analysis showed there was systematic missingness in the data, we conducted sensitivity analyses to assess if the missingness posed a material threat to the study findings. This is described in further detail by aim in the following sections.

3.10 Aim 1 Statistical Analysis

First, we summarized important maternal baseline characteristics. We the estimated the unweighted and weighted cumulative incidence and risk differences (RD) of preterm birth at 37 weeks using the complement of the Kaplan-Meier estimator given exposure to a category of HIV treatment (Formula 1). Inferences are conditional on survival to 20 weeks gestation. The origin for each pregnancy was the date of enrollment or the date of first treatment in pregnancy, whichever occurred first, and the timescale was gestational weeks until end of pregnancy/delivery. There was no right censoring or loss to follow up because all pregnancies were followed until pregnancy end.

Formula 1: Kaplan-Meier estimator for cumulative incidence

$$\hat{F}^{KM}(t|\ HIV\ Treatment = z) = 1 - \prod_{k:R_k \leq t} \left\{1 - \frac{d_k(z)}{n_k(z)}\right\}$$

$\hat{F}^{KM}$ is risk of preterm birth as estimated by the complement of the Kaplan-Meier estimator. t is a given time; R_k is a time when at least one birth occurred; d_k is the number of births that occurred at time R_k; and n_k is the number of fetuses known to have survived up to time R_k. HIV treatment is the exposure of interest, and z is an exposure category within Z.

Time-to-event methods are appropriate for this research question because we wanted to estimate the risk of birth relative to advancing gestational age, accounting for both late entry and time-varying exposure. Pregnancies could contribute gestational weeks to any of the exposure groups depending on their exposure status at any given time during gestation. Exposure-

switching is handled by censoring a pregnancy at the time of treatment switching; the observation time then moves to the treatment to which she switched. To estimate the risk and risk difference of PI cART compared to both no treatment, ZDV monotherapy and NNRTI cART, we used the cumulative incidence at 37 weeks, 32 weeks and 28 weeks to calculate absolute risk differences, using PI cART as the common reference. Weighted estimates reflected the changing treatment regimens over gestation and to produce a visual representation of the changing risk over time, accounting for confounding. The visual representation of the weighted risk curve is the counterfactual scenario had pregnancies been exposed to one treatment throughout gestation.

We used stabilized IPW to control for confounding. If confounders had missing values, we identified important predictors of missingness and included them in a pooled logistic regression model with missinginess as the outcome. We then used the resulting model parameters to generate a stabilized IPW for missingness. This missingness weight was then applied to a multinomial logistic regression model (the propensity score model) to generate a stabilized IPW for the confounder with missing values. If a confounder did not have missing values, we directly estimated stabilized IPW for treatment using a similar propensity score model as described previously. The final analysis weight was the product of the missingness and treatment weights.

Since we utilized nonparametric methods to estimate risk, we generated 95% confidence intervals for risk and risk difference estimates using 200 bootstrapped samples (with replacement). We arrived at the number of bootstrapped samples by considering the distribution and variance of the bootstrapped effect estimates. We also conducted sensitivity analyses to

randomly choose one pregnancy for inclusion into the analysis sample if a woman contributed more than one eligible pregnancy to see if the effect estimates materially changed.

3.11 Aim 2 Statistical Analysis

To estimate causal effects, we designed our analysis for the emulated trial to be comparable to that to the target trial. We first summarized maternal baseline characteristics stratified by exposure group. We then used a log-Poisson model to approximate the log-binomial model in order to estimate risk ratios (RR) for the effect of ART on preterm birth, for both the conditional and unconditional analyses (Formula 2).[73] A Poisson model was used because the adjusted log-binomial model failed to converge.[74] Generalized estimating equations (GEE) with an exchangeable correlation matrix were used to generate 95% confidence intervals. We accounted for confounding by including important baseline confounders to emulate randomization in the statistical model.

Formula 2: Poisson regression model

$$logP(\text{Preterm Birth} = 1) = \alpha + \beta_1(ART\ Exposure) + \beta_2 X_2$$

Where X is a matrix of potential covariates in the final adjustment set.

For the emulated target trial analysis, RRs were estimated for the effect of baseline ART on preterm birth, comparing each exposure group to PI cART as the reference. We conducted two analyses, one assuming that exposure was unconditional (like a true RCT) and another analysis assuming that exposure was conditional on baseline covariates (a more realistic scenario since these data are drawn from an observational cohort.) We identified important baseline covariates using the target trial eligibility criteria and analyzed a DAG to identify a minimally sufficient adjustment set (Appendix A).

To better understand the scope of missingness in the data, we summarized baseline covariates stratifying by complete and incomplete cases. While we had complete exposure and

outcome data, there were several baseline confounders we included in our multivariate models which had missing values and a complete case analysis would dispense of more than 50% of the sample. We therefore used MI for each analysis separately to impute missing covariate values.[75] We used fixed chain equations and included predictors of missing values that were used in the analytic models for the main effect, as well as auxiliary variables to improve the overall fit of the imputation model.[76] We then generated 30 imputed datasets for each analysis and ran the multivariate analysis model on each imputed dataset. Rubin's rules was used to pooled the resulting parameters to generate the adjusted risk ratios and 95% confidence intervals.[77]

3.12 Tables and Figures

Table 3.1. Subject Typology based on CD4 thresholds, and timing of treatment initiation, 1996 to 2005.

Type	Pregnancy-related Treatment Initiation	Description	Conception (0 weeks)	1st Trimester (18±2 weeks)	2nd Trimester (25±2 weeks)	3rd Trimester (34±2 weeks)	Delivery (Any time)
			Pre-Enrollment	Visit 1	Visit 2	Visit 3	Delivery
1	Preconceptional User *(EXCLUDED)*	Likely initiated prior to conception, with CD4 < 200 or 350	✓	✓	[✓]	[✓]	[✓]
2	Preconceptional User *(EXCLUDED)*	Likely initiated prior to conception, with CD4 < 200 or 350	[✓]	o	✓	[✓]	[✓]
3	New Initiator (ART Unexposed at Conception)	Initiated before 28w irrespective of CD4	o	✓	[✓]	[✓]	[✓]
4	New Initiator (ART Unexposed at Conception)	Initiated before 28w irrespective of CD4	o	o	✓	[✓]	[✓]
5	New Initiator (ART Unexposed at Conception)	Initiated after 28w irrespective of CD4	-	o *or* -	o	✓	[✓]
6	No ART during pregnancy	No ART or Prophylaxis only, likely missed/ asymptomatic	-	-	-	o	✓

✓*indicates treatment is known; "*[✓]*" indicates treatment is assumed based on data collected; "o" data are missing even though subject is being observed; "-" indicates data are not available because subject has not entered the study.*

Table 3.2 Measured covariates considered for analysis for Aims 1 and 2.

	Variable Name	Definition	Frequency of measurement	Method of Measurement
1	Gestational Age	Time since conception in weeks	Each study visit including delivery	Subject recall of last menstrual period, Fetal Ultrasound
2	Maternal Race/Ethnicity	White, Black, non-White Hispanic, American Indian, Asian, and Other	Enrollment	Self-report
3	Maternal Weight	In kilograms	Each study visit	Measured using a standard scale; weight at enrollment was included in analysis
4	Maternal Age	Years since birth	Enrollment	Calculated from Birth Year
5	Tobacco/Alcohol Use During Pregnancy	Ever/Never using Tobacco or Alcohol during current pregnancy	Each study visit	Self-report
6	Marijuana/ Heroin/ Crack /Cocaine/ Intravenous Drug Use During Pregnancy	Ever/Never using any illegal substances during current pregnancy	Each study visit	Self-report and urine test
7	CD4 Count	Continuous measure expressed as the number of CD4 T lymphocyte cells per cubic micromilliliter (mm^3) of blood. HIV infection targets primarily CD4 cells; HIV treatment promotes immune recovery, indicated by increasing CD4 levels once treatment is initiated.	Collected at each study visit, medical abstraction	Collected blood samples, upon which flow cytometry was conducted at laboratories certified by the NIAID Immunology Quality Assurance Program (Adult AIDS Clinical Trials Group, 2006a)
8	Viral Load	Continuous measure of the number of copies of HIV RNA per milliliter of blood. The goal of HAART is to suppress viral load to "below detection" by the test; viral load values <400 are considered undetectable for the proposed study. [78]	Collected at each study visit, medical abstraction	

9	Diabetes During Pregnancy	Categorical, Yes/No	Confirmed at last visit prior to delivery, corresponding to 28 or 34 week visit	Measured by glucose tolerance test.
10	AIDS-defining illness during pregnancy	Categorical, Yes/No	Collected at each study visit, medical abstraction	
11	Hypertension During Pregnancy	Categorical, Yes/No	Confirmed at last visit prior to delivery, corresponding to 25 or 34 week visit	Blood pressure reading in excess of 140/90 mm Hg and either a blood test/ urine analysis/ fetal ultrasound/ nonstress test confirming pre-eclampsia.
12	Infection During Pregnancy (trichomonas/ bacterial vaginosis)	Categorical, Yes/No	Collected at each study visit	Standardized laboratory testing to determine whether woman was infected with bacterial vaginosis, trichomonas, or syphilis.
13	Cohort time	Describes the time during which the pregnancy occurred during follow-up, categorized into the following: prior to 2/28/1994; 3/1/1994 to 7/31/1996; and after 7/31/1996 corresponding to HIV treatment eras.	Enrollment	Based on time of enrollment

CHAPTER 4: AIM 1 - PROTEASE INHIBITOR-CONTAINING ART REGIMENS AND PRETERM BIRTH: A REEXAMINATION USING SURVIVAL ANALYSIS METHODS

4.1 Introduction

Worldwide, preterm birth is associated with 75% of perinatal mortality and more than half of long-term morbidity of children, including cognitive disability, poor motor skills, behavioral problems, hearing loss and chronic lung disease.[33–36] The causes of preterm birth are complex, particularly among women living with HIV. Women living with HIV in the United States are at high risk for preterm birth (PTB) not only due to HIV infection itself,[79] but also because they experience other preterm birth risk factors like gestational diabetes and gestational hypertension; [3,38] alcohol and drug use;[37,39] and smoking cigarettes both prior to and during pregnancy at higher rates than women without HIV infection.[40]

An increasing number of HIV-infected women of reproductive age are initiating antiretroviral therapy (ART) worldwide. Because of this, the number of women on treatment before and during pregnancy is increasing.[37,80] Antiretroviral therapy (ART) significantly reduces the risk of HIV transmission from mother to child, however several studies suggest that ART can cause preterm birth.[48,81] Understanding the possible consequences of HIV treatment on preterm birth is therefore of urgent importance in the growing population of reproductive-aged women living with HIV.

Several studies investigating the relationship between ART and preterm birth have found that regimens containing protease inhibitors (PI) are associated with preterm birth.[43,48,81–83] However, other studies show no differences in preterm birth occurrence by ART regimen. [57,84,85]

Reconciling these conflicting findings has been a research challenge over the past decade. Controlling HIV infection through ART minimizes the inflammatory response to systemic infection, thereby lowering preterm birth risk.[86,87] Use of ART in pregnancy would therefore be expected to reduce, rather than increase, the risk of preterm birth.[54,55] Even so, it is possible that certain ART drugs – while preventing transmission of HIV – induce inflammation through immune reconstitution that could cause premature initiation of the labor cascade.[38] There is evidence that Lopinavir and Nelfinavir – both PIs – show declining bioavailability in the third trimester compared to levels prior to pregnancy.[88,89] This means that viral suppression may not be maintained in later gestational weeks, and could cause a reemergence of infection-associated inflammation and the accompanying risk of preterm labor onset.[90] PIs are also associated with adrenal dysfunction and metabolic disturbances (i.e. diabetes, hyperglycemia) in pregnant women, which are also associated with preterm birth.[91–93]

Studies showing PI-based regimens to be harmful may not have sufficiently accounted for confounding by indication, given that PI-based regimens were often reserved for individuals with more severe disease.[94,95] Another explanation for the discrepancies could be the methodological flaws in analyzing data from observational HIV pregnancy cohorts. Treatment misclassification caused by intent-to-treat approaches[96] or conditioning on future exposures,[63] and selection bias induced by excluding untreated pregnancies are common analytic limitations in the HIV and pregnancy literature.[56] Additionally, studies in the current literature tend to not show the changing risk of preterm birth in pregnancy, which is important in understanding how exposures affect pregnancy and birth during gestation.

The primary objective of this study is to reexamine the effect of PI-containing combination ART (cART) on the risk of preterm birth using methods that account for important

facets of observational pregnancy cohorts. We estimated the risk of preterm birth by ART regimen using the complement of the Kaplan-Meier estimator which accommodates treatments that change during observation (time-varying exposure). Given that these data are derived from an observational context, the use of survival analysis methods also allows us to account for late entry into the cohort and to examine the changing risk of birth over the entire gestational period by exposure to offer further context pertaining to the reported differential effects of PI ART compared to other regimens on preterm birth.

4.2 Methods

We used publicly available data from the Women and Infant Transmission Study (WITS). WITS was a US-based, multi-center, prospective, interval cohort designed to study the natural course of maternal-infant HIV-1 infection between 1990 and 2005. [1,2] Prospective data collection included structured patient interviews and standardized clinical and laboratory assessments at each study visit. Retrospective data collection consisted of medical record abstraction. WITS study sites were hospitals located in Boston, MA; Worcester, MA; New York, NY; Houston, TX; San Juan, PR; and Chicago, IL. The entire study population was confirmed to be HIV positive at the enrollment visit. Participants were tested using enzyme-linked immunosorbent assays (ELISA), with subsequent HIV diagnosis confirmation using Western Blot. Informed consent was obtained for all women and infant participants according to each site's local institutional review board, following federal guidelines for research. Study visits coincided with antenatal visits and all prenatal care was at the discretion of the clinician. This is most important when understanding the circumstances of treatment decision-making (e.g., timing, switching and regimen type), as well as labor and delivery-related decisions (e.g., labor induction, methods of assisted delivery, cesarean-section). Additional study details for the WITS parent study are published elsewhere.[1,2]

While questions pertaining to the association of ART and preterm birth have been previously explored in WITS,[2] this study differs from past work in the following ways: First, we estimate risk differences of preterm birth by exposure while accommodating late-entry into the cohort and time-varying exposure by utilizing survival (time-to-event) methods instead of conventional binary methods (e.g., logistic or log-binomial regression). Binary methods are useful when both the observation time and exposure plans are known and fixed. Observational settings are by definition non-interventional, meaning the observation time and exposure plans are both variable. In such settings, survival methods flexibly accommodate late-entry, time-varying exposure and censoring, while binary methods do not account for these elements as easily**.** Survival methods also provide additional context that is often lacking in the available literature describing ART and birth outcomes by visualizing events over time. Second, we analyze a subset of WITS pregnancies occurring between 1996 and 2005 i.e. in the era of combination regimens known as highly-active antiretroviral therapy. Restricting the analysis to the current treatment era facilitates the results to be more relevant in the current clinical context. (We will refer to combination regimens in this paper as cART.) Third, we utilize a causal diagram[97] to guide final covariate adjustment to facilitate interpretation of results based on substantive evidence, in contrast to stepwise covariate elimination or other related, statistics-driven methods.

4.3 Study Population

We included singleton pregnancies occurring between 1996 and 2005 among women living with HIV (aged 15 – 45) that survived past 20 weeks gestation and enrolled prior to 28 weeks gestation. We conditioned on fetal survival to 20 weeks to remove the possibility of miscarriage as a competing event. (Figure 4.1) Pregnancies enrolling at points B or C were eligible for analysis. Pregnancies unexposed to ART at the enrollment visit were included, under

the assumption that those unexposed at enrollment were likely unexposed to ART at the time of conception. Gestational age (GA) was estimated for each pregnancy at entry, at each study visit, and at delivery. GA and length of pregnancy were calculated according to agreement between last menstrual period (LMP) as reported by the mother and first available ultrasound.[72] In cases of disagreement between these two measures, GA was determined using ultrasound only and/or neonatal physical examination.

4.4 Outcome Classification

Pregnancies ending in either live birth or stillbirth (hereafter referred to as "birth" or "delivery") between 20 weeks and 36 6/7 weeks were considered preterm. We included stillbirths in the outcome of interest because it is an adverse outcome that could plausibly be caused by ART exposures. All stillbirths in the analytical sample occurred prior to 37 weeks gestation. Secondary outcomes of interest were very preterm birth (< 32 weeks) and extremely preterm birth (<28 weeks).[98] Births occurring on or after 37 weeks were considered term.

4.5 Exposure and Confounder Classification

Timing and type of exposure were ascertained at each study visit through structured patient interviews, medical chart review and retrospective medical record abstraction when available. Treatment data were recorded as single drugs and the corresponding start and stop of exposure expressed as months since enrollment. At the enrollment visit, the clinician either prescribed ART or allowed pregnancies to proceed without treatment until the next visit, when the treatment plan could be reconsidered. We were unable to confirm if women were ART-naïve at enrollment due to incomplete data on treatment history and timing of HIV diagnosis. Exposure to a regimen for less than two weeks was considered too short of a window to have any impact on birth outcomes.[99] Consequently, the exposure was removed and exposures on either side of the <2 week window equally closed the gap.

ART exposures considered in this analysis are regimens comprised of either a single drug or combination of drugs drug classes prescribed at a given time. Exposure regimens considered in this study are PIs; nucleoside reverse transcriptase inhibitors (NRTIs); and non-nucleoside reverse transcriptase inhibitors (NNRTIs). Exposure categories are defined as the following four mutually exclusive groups: no therapy, Zidovudine (ZDV) monotherapy; PI-based cART; NNRTI-based cART; and "mixed" therapies defined as including both PIs and NNRTIs prescribed concurrently. ZDV monotherapy was the standard of care for pregnant women during the investigated timeframe and was often prescribed concurrently with cART.[22] When both ZDV and cART were prescribed together, women were categorized as exposed to cART. (Appendix B).

Baseline information about each pregnancy was collected at the enrollment visit through standardized enrollment questionnaires. Race/ethnicity was classified into four racial categories (American Indian or Alaskan Native, Asian or Pacific Islander, Black, and White*)* and two ethnic categories (Hispanic origin, and Not of Hispanic origin) as per the 1996 U.S. Census Bureau. Racial and ethnic categories were combined into a single race/ethnicity category in the WITS as White, Black, Hispanic and other (including Native American/Alaskan Native and Asian/Pacific Islander). Body mass index (BMI) was calculated using weight and height at enrollment visit. The following covariates were all coded dichotomously as yes/no: history of diabetes and hypertension; use of cigarettes, marijuana, crack/cocaine, heroin and alcohol in pregnancy; coinfection with syphilis or chlamydia during pregnancy; and presence of AIDS-defining illness. For this study, access to prenatal care described whether a pregnancy was exposed to at least one prenatal visit prior to 28 weeks gestation. Baseline viral load measurements were taken at

enrollment, corresponding to either the 12- or 24-week appointment. Baseline viral load and maternal age were modeled continuously as restricted quadratic splines with four equal knots.[100]

We used a DAG to identify a minimally sufficient adjustment set of confounders. The final adjustment set included access to prenatal care, baseline viral load, baseline maternal age, AIDS-defining illness and study site. These confounders are accounted for through restriction (access to prenatal care) and standardization (baseline viral load and maternal age). Only three pregnancies had an AIDS-defining illness so this confounder was removed from the adjustment set. Study site was also not considered in the analysis because this variable was removed from public-use dataset to preserve the confidentiality of study participants. Prior preterm birth is often adjusted for in analyses of exposures and preterm birth but we excluded this from the DAG.[101] Prior preterm birth is a proxy for other underlying biological factors causing preterm birth, but prior preterm birth itself is unlikely to have a causal link to the exposure of interest or timing of delivery.

4.6 Statistical Methods

We used inverse-probability-of-exposure weighted risk curves using the complement of the Kaplan-Meier estimator to estimate cumulative incidence and risk differences of preterm delivery by time-varying exposure. PI cART was the reference group for all risk difference measures. In these analyses, each pregnancy was followed from the week of enrollment until the week of delivery. There was no right-censoring or lost-to-follow up because all pregnancies were followed until the end of pregnancy.

Controlling for access to prenatal care was done through sample restriction. Stabilized inverse probability weights (IPW) were used to account for missing baseline viral load values, as well as to control for confounding by baseline viral load and maternal age. The numerator and denominator of the missingness weights were calculated using logistic regression and included

maternal age at enrollment, baseline ART, race/ethnicity and gestational age at study entry as predictors. We calculated treatment weights for viral load and maternal age using pooled multinomial logistic regression. The missingness weight was included in the treatment weight calculation for viral load. The final analysis weight was the product of the IPWs for missing baseline viral load, confounding by viral load and confounding by maternal age. We accounted for tied event times using the exact method. Confidence intervals for risk differences were calculated using the standard deviation from a bootstrap of 200 samples (with replacement) from the observed data.

Pregnancies were allowed to switch exposure categories in gestation. In the crude Kaplan-Meier context, the pregnancy was censored at each point of exposure-switching and continued on another curve corresponding to the new exposure. The weighted time-varying exposure Kaplan-Meier risk curves illustrate the counterfactual scenario had pregnancies been exposed to only one treatment from enrollment to delivery, accounting for measured confounding using weights. Exposure contrasts comparing regimens containing both PIs and NNRTIs ("mixed therapies") to regimens of interest are not reported in this paper since it is a highly heterogeneous exposure group, and comparisons to it are not useful to future policy. We were unable to exclude pregnancies exposed to mixed therapies entirely. Several pregnancies were exposed to both mixed therapies and exposures of interest, and it was necessary to account for all changing treatment plans in the time-varying exposure analysis.

We conducted two sensitivity analyses to explore whether risk estimates changed materially under the following scenarios. Because the Kaplan-Meier estimator does not account for clustered data, we randomly selected one pregnancy for inclusion from each woman

contributing more than one pregnancy to the analysis sample. We also excluded pregnancies with missing singleton status.

4.7 Results

Of the 3,297 pregnancies included in the WITS parent study, 1,270 were excluded because they were enrolled prior to 1996. Among the remaining pregnancies, 48 were excluded because they were multiple gestation, 765 were excluded because the pregnancy ended prior to 20 weeks gestation or were enrolled after 28 weeks gestation, and 147 were excluded because the pregnancies were exposed to ART at conception. Therefore, a total of 1,067 HIV-positive pregnancies in 932 women were followed until delivery (Figure 4.2).

Enrollment in the WITS analysis sample occurred between 12 and 21 weeks gestation (median 17 weeks). Approximately 42% of women included in the analysis sample were Black (N = 446) and a third were Hispanic (N = 359). Fifty-three percent of women were between the ages of 25 and 34 (N =569), and the median BMI at enrollment was 26.9. Drug and alcohol use in pregnancy was common in this population, with 31% reporting use of cigarettes (N=329), 20% reporting alcohol consumption (N = 213) and 20% using hard drugs (N = 211). (Table 4.1).

Twenty-four per cent of pregnancies had viral load <400 copies/ml^3 indicating viral suppression at enrollment (N = 236). All pregnancies were unexposed to ART at conception, of which over half remained on no therapy beyond the enrollment visit (N = 545). At the enrollment visit, 22% of pregnancies were prescribed PI cART (N = 232), 20% were started on ZDV monotherapy (N = 216) and 3% were prescribed NNRTI cART (n = 32). Total observed gestational weeks contributing to effect estimates for PI cART was more than seven times that for NNRTI cART (6778 weeks versus 940 weeks). By the end of follow-up, 29% of pregnancies had switched treatment regimens at least once (Table 4.1). Thirteen percent of deliveries occurred in week 37 (N=137) and 29% occurred in week 38 (N = 304) (Figure C.1). The median

gestational age at delivery was 38 weeks, and 18% were delivered prior to 37 weeks (N = 192) (Figure C.2).

As expected, risk of preterm birth (<37 weeks) was highest among pregnancies exposed to no therapy (risk: 23%; 95% CI: 19%, 26%). After adjusting for confounding, the risk of preterm birth was comparable between no therapy and ZDV monotherapy at approximately 20%, followed by PI cART at 17% and NNRTI cART at 7% (Table 4.2 and Figure 4.3A). The unweighted preterm birth risk for NNRTI cART was 12 percentage points lower than that for PI cART (RD: -12%; 95% CI: -18%, -6%). After applying inverse probability weights to correct for missing data and confounding, the difference was attenuated slightly to 10 percentage points (RD: -10%; 95% CI: -21%, 2%), though the confidence interval remained wide and included the null value (Table 4.2 and Figure 4.3A). We found there to be no meaningful difference in risk of preterm birth between PI cART and ZDV monotherapy in either the unweighted [RD: 0%; 95% CI: -3%, 4%] or weighted [RD: 3%; 95% CI: -2%, 8%] estimates.

In examining deliveries occurring very preterm (<32 weeks), exposure to PI cART was associated with the lowest preterm birth risk while exposure to no therapy was associated with the highest risk in the unweighted and weighted analyses. (Table 4.2 and Figure 4.3B). There was a significant difference in weighted risk estimates comparing both No Therapy (RD: 3%, 95% CI: 1%, 6%) and ZDV monotherapy to PI cART (RD: 3%, 95% CI: 1%, 5%). The incidence of birth prior to 28 weeks was very low in this cohort (indeed, no women exposed to NNRTI cART delivered prior to 28 weeks), thus we were unable to make meaningful comparisons around this gestational age cut-point.

The unweighted risk curves show the crude time-to-delivery by exposure, allowing for changing exposure over gestation (Figure 4.4). The weighted time-varying exposure risk curves

(Figure 4.5) stratified by exposure show separation between 20- and 35-weeks with no therapy and ZDV monotherapy exposures showing a higher proportion of deliveries in earlier gestational weeks than both cART regimens. While PI cART risk curve is higher than the NNRTI cART curve throughout gestation, the curves cross at week 34.

None of the above results were materially changed by restricting the sample to a single pregnancy per woman in sensitivity analyses**.** Thirty-six pregnancies had missing data confirming singleton status (3.4%), but including them did not significantly affect risk estimates either. Results from the sensitivity analyses are shown in Appendix C.

4.8 Discussion

As expected, combination ART therapies are not associated with increased risk of preterm birth compared to ZDV monotherapy, as presented in the original WITS publication.[3] The effect estimates for very preterm birth and the risk curves produced by the complement of the Kaplan-Meier estimator confirm that suppressive regimens confer protection against preterm births at lower gestational ages in contrast to pregnancies exposed to monotherapy and no treatment, and continue to have a protective effect throughout gestation. Non-suppressive regimens are no longer prescribed or clinically relevant due to evolving HIV treatment guidelines.[102]

Results presented in Figure 4.3 suggest that PI cART, while protective overall compared to no ART, is relatively harmful when compared to NNRTI cART, consistent with several other studies.[59,95,102,103] However, Figure 4.5 shows the weighted risk curves crossing between 32 and 37 weeks, calling into question that PI cART is definitively harmful. These results show that even if preterm birth risk is reported at 37 weeks and 32 weeks, the risk could change between these two time points, offering important context to the overall effect of ART on preterm birth risk. In these data however, it is more likely that the risk curves crossing is due to the small

sample size of NNRTI-exposed pregnancies. Having small sample sizes of NNRTI-exposed pregnancies compared to PI-exposed pregnancies is common among observational pregnancy cohorts, further lending to the challenges in understanding the comparative risk of preterm birth between PI- and non-PI- containing regimens.[59,104,105]

The numerous studies implicating PI cART utilized binary regression to report odds ratios or risk ratios at gestational weeks consistent with conventional delivery-timing classification at 28-, 32- and 37 weeks. Because our study employs non-parametric survival methods to estimate risk, we are able to report risk both in terms of delivery-timing classifications, and as a dynamic risk function by exposure over time as shown in Figures 4.4 and 4.5. The risk curves support better interpretation of risk over time, apart from the rigid preterm birth classifications. In our study, cumulative incidence of preterm delivery at 37 weeks among PI cART-exposed pregnancies was higher than those exposed to NNRTI cART, but NNRTI cART risk estimates had undesirably wide confidence intervals containing the null value. This is likely because pregnancies exposed to NNRTI cART contributed the least number of person-weeks to the overall time at risk for delivery and only two births occurred during NNRTI cART exposure. At the time of the study, PI-based regimens were more frequently prescribed than NNRTI- regimens because there were far more PI drugs approved by the Federal Drug Administration.

This study was limited in ways similar to those of many observational HIV pregnancy cohorts. First, enrollment in WITS was limited by the timing of pregnancy detection and linkage to antenatal care. Ideally, pregnancies would have been enrolled from conception (or before)[106] with complete ART treatment history information from HIV diagnosis. Without complete HIV diagnosis and treatment history, we were unable to definitively confirm timing of ART initiation

as it related to conception. Both nadir CD4 count (typically the CD4 count at HIV diagnosis) and timing of treatment can affect risk of preterm birth among pregnant women living with HIV. The available dataset did not include study site, so were unable to account for site-specific differences in prescribing patterns and severity of disease.

Second, measurement error across a few key variables could be a source of bias in this analysis as well. Challenges in measuring gestational age accurately and the accompanying bias in its measurement are well-documented in the literature, and we suspect this bias to be present in our study as well.[107,108] More than 40% of all deliveries occurred in weeks 37 and 38, just missing the preterm birth cutoff (Figure C.1). It is likely that the timing of exposure to ART regimens could potentially be a source of bias in this analysis as well. Actual dates are not provided in the dataset; instead, the usage periods are bounded by "time from enrollment in months." For records only containing the month and year, days were imputed as "15". Further, our time-varying exposure analysis assumes that there is no cumulative effect of the exposure on the outcome. Even though our analysis did not examine the effect of exposure duration on preterm birth per se, estimates should still be interpreted with caution due to the lack of reliable start and stop dates for treatment. Third, our analysis assumes no unmeasured confounding - strong assumptions that may not be valid. However, we considered a host of potential confounders based on an extensive review of the literature, and using causal diagrams included those which were causally important.

A primary strength of this study is demonstrating the use of nonparametric survival methods to show changes in preterm birth risk over time. Preterm birth risks estimated using regression and reported at one or two cut-points can obscure changes in risk at intervening gestational ages which could be potentially important. Further, this method accounts for

changing exposures over the risk period, approaching a per-protocol analysis. While left truncation bias is a challenge in virtually all pregnancy cohort studies (including ours since we allow for late-entry between 20 and 28 weeks) our study mitigates the bias by restricting to pregnancies unexposed to ART at conception and surviving to 20 weeks. We also report effects in absolute terms which are easily interpretable.

The challenge in reaching a clear understanding of the causal effect between ART and preterm birth is in part due to the limitations in comparing findings across many observational studies employing a wide range of methodological approaches. New observational studies should consider enrolling larger numbers of preconceptional women to better estimate time of conception and ascertain a better estimate of the total number of pregnancies at risk. Analyses of data from observational cohorts should consider survival methods as an alternative to binary regression to more flexibly accommodate the realities of changing treatments in observational contexts, and to facilitate ease of interpreting findings within and across studies. Additional analysis options for observational pregnancy cohorts could also include target trial approaches,[69] which offer the opportunity to make clear causal claims in the absence of randomized control trial data. Data collection for this analysis pre-dated the introduction of the integrase strand transfer inhibitors (INSTIs) class of drugs, but the methods we used in this paper would be particularly relevant as studies are now increasingly examining the effect of INSTIs on preterm birth risk compared to other ART regimens. In the absence of randomized evidence, it is important that researchers consider using methods like those demonstrated in this study to appropriately address the potential sources of bias common to observational studies of pregnancy, and to generate effect estimates that are useful to policy and clinical practice.

4.9 Tables and Figures

Figure 4.1. Survival analysis framework detailing subject eligibility, exposure and outcome relative to gestational weeks for Aim 1.

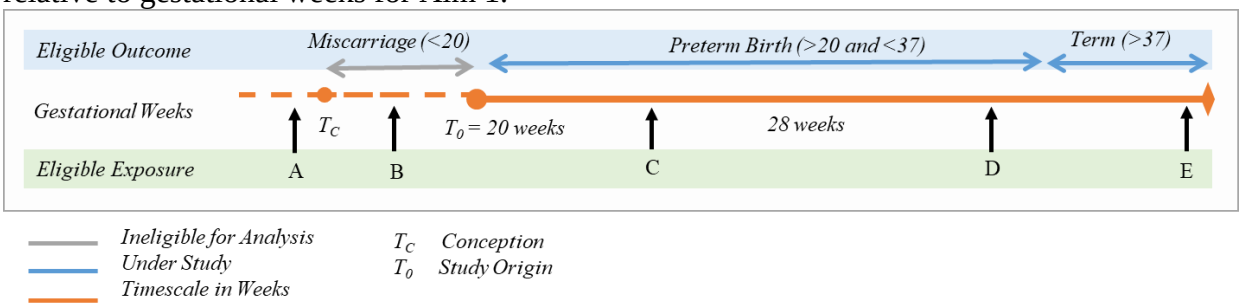


Figure 4.2. Consort diagram of pregnancies eligible for analysis from the Women and Infants Transmission Study (WITS).

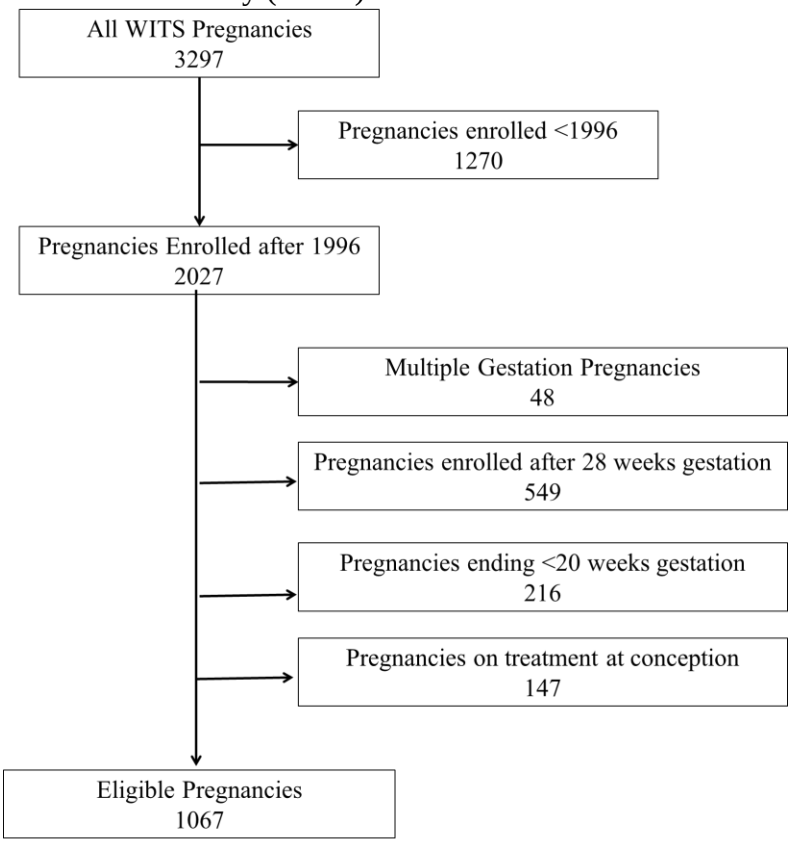


Table 4.1. Baseline characteristics of 1067 singleton pregnancies from 932 women living with HIV enrolled between 1996 and 2005 in WITS.

Baseline Characteristic	N	Value [% or Mean (IQR)]
Total Number of Women	932	
Pregnancies in the study from the same woman		
1	813	87.2%
2	106	11.4%
3+	13	1.4%
Race/Ethnicity		
White	91	8.5%
Black	446	41.8%
Hispanic	359	33.6%
Other	21	2.0%
Unknown	20	1.9%
Total Number of Pregnancies	1067	
Maternal Age		
18-24	355	33.3%
25-34	569	53.3%
35-49	143	13.4%
Baseline BMI	752	26.9 [23.2, 31.4]
Missing	315	29.5%
Baseline BMI		
Less than 19.8	29	2.7%
19.8 - 26.0	302	28.3%
Greater than 26.0	421	39.5%
Missing	315	29.5%
Substance Use During Pregnancy		
Cigarettes	329	30.8%
Missing	4	0.4%
Alcohol	213	20.0%
Missing	3	0.3%
Marijuana	117	11.0%
Missing	11	1.0%
Hard Drugs	211	19.8%
Missing	3	0.3%
Gestational Age at Study Entry (Weeks)	1067	17 [12, 21]
CD4 at Enrollment	897	425 [289, 605]
Missing	170	15.9%
Viral Load at Enrollment	798	2134 [0, 15000]
Missing	269	25.2%
Virally Suppressed at Enrollment (<400 copies/ml[3])*	255	23.9%
Missing	269	25.2%

Baseline Characteristics (continued)	N	Value [% or Mean (IQR)]
ART Regimen Status at Enrollment		
No Therapy	545	51.1%
PI cART	232	21.7%
NNRTI cART	32	3.0%
ZDV Monotherapy	216	20.2%
Other/Mixed Therapy	42	3.9%
Observed gestational weeks by regimen (N = 22246)		
No Therapy	7210	32.4%
PI cART	6778	30.5%
NNRTI cART	940	4.2%
ZDV Monotherapy	6010	27.0%
Other/Mixed Regimen	1308	5.9%
Switching Regimen During Pregnancy	311	29.1%
HIV Transmitted to Child	21	2.0%
Missing or Inconclusive	111	10.4%
Pregnancy Outcome		
Live Birth	1048	98.2%
Still Birth	13	1.2%
Spontaneous Abortion	6	0.6%
Gestational Age at Delivery	1067	38.0 [37.0, 39.0]
Gestational Age at Delivery		
Term (>37 weeks)	875	82.0%
Preterm (20 - 36 weeks)	192	18.0%
Late Preterm (32 - 36)	146	13.7%
Very Preterm (28 - 32)	23	2.2%
Extremely Preterm (20 - 28)	23	2.2%
Mode of Delivery		
Elective C-Section	266	24.9%
Non-Elective C-Section	131	12.3%
Assisted Vaginal Delivery	27	2.5%
Spontaneous Vaginal Delivery	460	43.1%
Vaginal Delivery (assisted status unknown)	10	0.9%
C-Section (elective status unknown)	48	4.5%
Delivery mode unknown	4	0.4%
Missing	121	11.3%

*Viral suppression was defined as (<400 copies/ml[3]) at the time of study enrollment; the current definition is (<50 copies/ml[3])

Table 4.2. Unweighted and weighted risks and risk differences for the effect of ART regimen on preterm delivery among pregnancies enrolled in WITS between 1996 and 2005 from women living with HIV (aged 15 to 45) accounting for time-varying exposure with bootstrapped 95% confidence intervals (N = 1067).

	Unweighted						Weighted*			
	Births	**Censored**	**Risk**	**95% CI**	**RD**	**95% CI**	**Risk**	**95% CI**	**RD**	**95% CI**
Preterm (<37 Weeks)										
PI cART	62	6	0.16	(0.14, 0.19)	0.	0.	0.17	(0.14, 0.20)	0.	0.
No Therapy	64	295	0.23	(0.19, 0.26)	0.06	(0.02, 0.10)	0.21	(0.17, 0.26)	0.05	(0.00, 0.10)
NNRTI ART	2	0	0.05	(0.00, 0.10)	-0.12	(-0.18, -0.06)	0.07	(-0.04, 0.18)	-0.10	(-0.21, 0.02)
ZDV Monotherapy	56	8	0.16	(0.14, 0.19)	0.00	(-0.03, 0.04)	0.20	(0.16, 0.24)	0.03	(-0.02, 0.08)
Very Preterm (<32 Weeks)										
PI cART	9	5	0.02	(0.01, 0.03)	0.	0.	0.02	(0.01, 0.04)	0.	0.
No Therapy	22	260	0.06	(0.04, 0.08)	0.04	(0.02, 0.06)	0.06	(0.03, 0.08)	0.03	(0.01, 0.06)
NNRTI ART **	0	0	0.00	(0.00, 0.00)	NA	NA	0.00	(0.00, 0.00)	NA	NA
ZDV Monotherapy	14	6	0.04	(0.03, 0.05)	0.02	(0.00, 0.04)	0.05	(0.03, 0.07)	0.03	(0.01, 0.05)

*Weighted risks and risk differences account for baseline viral load and maternal age.
**Risk differences were not calculated because no events occurred prior to 32 weeks.

Figure 4.3. Unweighted and weighted risks and risk differences for the effect of ART regimen on (A) preterm delivery and (B) very preterm delivery among pregnancies accounting for time-varying exposure. Unweighted estimates are in lighter shades and weighted estimates are in darker shades, with bootstrapped 95% confidence intervals (N = 1067).

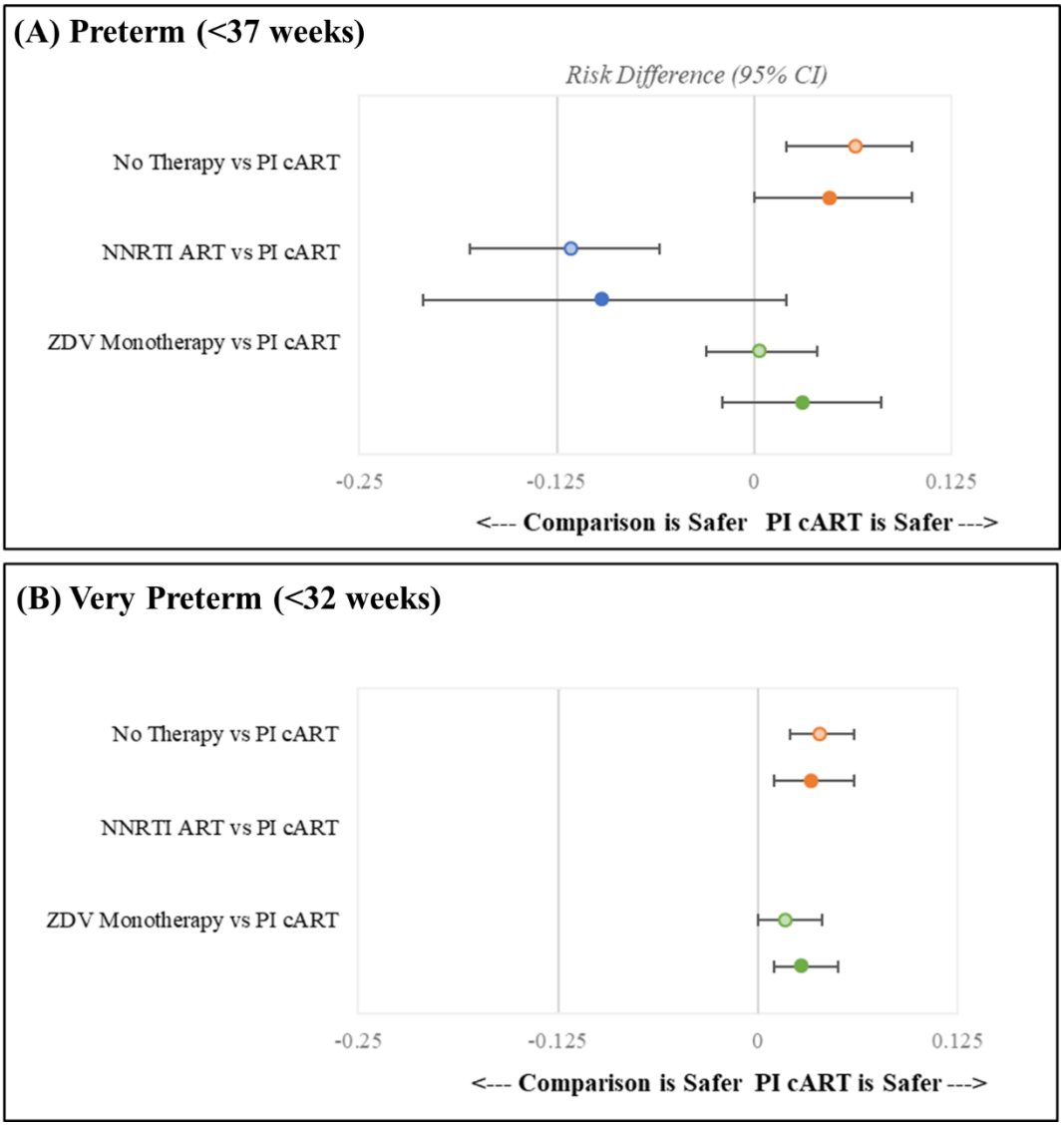


Figure 4.4. Cumulative incidence curves for delivery among singleton pregnancies enrolled in WITS between 1996 and 2005 from women living with HIV (aged 15 to 45) by time-varying exposure (N = 1067). Dashed lines appear at 20 and 37 weeks marking the preterm delivery period.

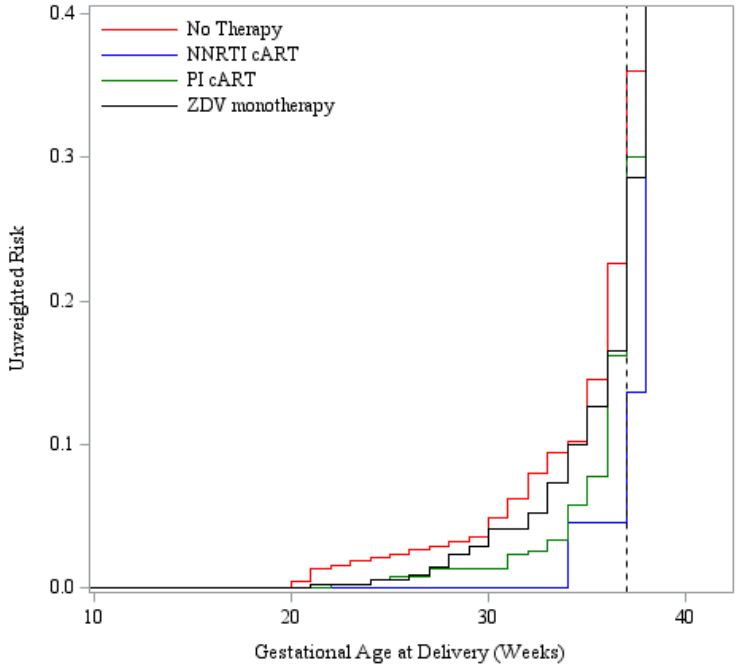
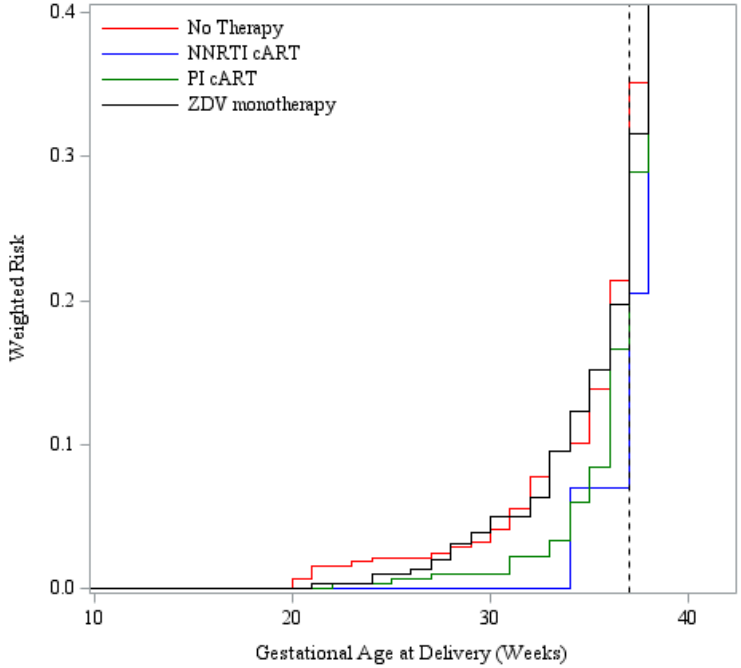


Figure 4.5. Cumulative incidence curves for delivery among singleton pregnancies, weighted for baseline viral load and maternal age.



CHAPTER 5: AIM 2 - EXPLICITLY EMULATING A TARGET TRIAL USING OBSERVATIONAL DATA: AN APPLICATION TO THE EFFECT OF ART IN PREGNANCY ON PRETERM BIRTH AND LESSONS LEARNED

5.1 Introduction

Randomized controlled trials are considered the gold standard to address causal questions about the effect of exposures on outcomes.[109] Often however, the ideal trial may not be feasible or ethical, particularly when the question involves human subjects. This is particularly the case when considering comparative effects among vulnerable populations, like pregnant women.[110] Groups are considered vulnerable when the ability to protect their interests and to provide informed consent is compromised in some way.[111,112] Pregnant women are fully capable of doing both, but because they are also responsible for protecting the health and interests of a growing fetus which cannot actively offer consent, there is a more complex calculus when weighing the risks and benefits of participating in medical research.

Medical research involving human subjects may be conducted in a variety of ways, broadly categorized as being either experimental and observational in design. The quintessential type of experimental design is the randomized controlled trial (RCT), where the investigator randomly assigns each study subject to receive an intervention and subsequently follows all subjects over a period of time to observe an outcome of interest.[113] Among many other applications, this design is the standard in pharmaceutical research to establish the safety and efficacy of new drugs coming to market, including antiretroviral therapy (ART) for treatment of HIV.

With the first successful placebo-controlled RCT demonstrating the effect of azidothymidine (AZT) against HIV in 1987,[114] the next three decades saw the introduction of over 25 antiretroviral drugs and drug combinations to market.[115] However, conventionally vulnerable populations, including pregnant populations living with HIV, have historically been under-represented in RCTs studying HIV treatment.[110,116] Ascertaining safety, teratogenicity, dosing and efficacy of ART drugs and regimens in pregnancy can therefore be challenging, and conducting an RCT including pregnant women living with HIV relies heavily on justifying true equipoise prior to randomizing treatment. More generally, RCTs can be prohibitively expensive and resource intensive; the costs and logistical burden of identifying study subjects, randomizing exposures, blinding investigators if applicable, documenting adverse events, coordinating additional safe-guards accommodating vulnerable populations, mitigating lost to follow up, etc. can be substantial. These considerations pose an additional barrier to studying pregnant women.[117]

When RCTs are not feasible due to ethical and practical considerations, pregnant HIV populations are frequently studied using observational cohorts. In contrast to RCTs, the investigators do not directly intervene on activities or exposures of the study subjects.[118] Rather, researchers observe a group of study subjects with varying levels of an exposure over time to examine the occurrence of one (or more) outcomes of interest.[113] Pregnant women living with HIV are identified and followed over time; their exposures, outcomes and other details are recorded usually through a combination of medical chart abstraction and/or study questionnaires at regularly scheduled clinical visits. This design offers a workaround when ethical and practical considerations pose challenges, but as with any study, the success of observational cohort studies depends heavily on the specific and appropriate definition of the study population, exposure and

outcome, as well as valid and consistent measurement of important covariates and confounders.[67,118] Not doing so means that the analysis and the resulting inferences will likely be limited.

The observational studies exploring the effect of ART type on preterm birth among HIV-positive pregnant women are limited in three primary ways. First, these studies are prone to selection bias. For example, several observational studies conducted in Europe, the United States, and sub-Saharan Africa have reported that preconceptional ART use is associated with increased risk or odds of preterm birth.[43,49,58–61] This finding lacks biological plausibility[54,55] and is likely due to the systematic exclusion of women who initiate ART after delivery.[51,56] Women initiating ART after delivery are often removed from the risk set (even if they experienced preterm birth) due to poorly defined inclusion criteria conditioning on exposure early in gestation, thereby biasing the study sample.[51] One way to address this type of bias is to include all fetuses at risk of the outcome – independent of initiation timing – in estimating risk.[62] By extension, cohort membership should be defined by exposure at baseline, however observational analyses of existing data often inadvertently violate this principle by using "future exposures" to define cohort membership at baseline, inducing both selection bias and possibly immortal person-time.[63] Trials mitigate the possibility of this sort of bias; the explicit, *a priori* definition of subject eligibility and the random assignment of exposure at the time of subject eligibility assessment help ensure that the exposure is independent from other factors.

Second, the inferences derived from observational cohorts can be limited by the use of inappropriate analysis methods. Many studies examining HIV and preterm birth are prospective or retrospective cohort studies, which utilize binary regression methods to estimate risk or odds ratios. The use of binary regression itself need not be wrong; rather the method assumes that

exposure is time-fixed, but this assumption is rarely made explicit when researchers report and interpret the effect estimate for the reader.[64] This is particularly relevant in pregnant populations when a woman can experience time in pregnancy being both exposed and unexposed to a treatment like ART, for example.[119] Further, women enrolled in a cohort typically have different start times, dependent on pregnancy detection and timing of the first prenatal visit. (RCTs by comparison, are specifically designed to start all subjects at the same "time zero.")[69] The possibility of having unobserved time at the beginning of pregnancy opens the door to competing risks (like miscarriage) not being accounted for in the analysis, or again, immortal person-time bias.[120]

Third, the effect estimates derived are not always useful to clinical decision-making. For example, logistic regression produces odds ratios. Odds ratios are very often reported in the literature but are hard to understand and interpret. Odds ratios are commonly understood and operationalized as risk ratios, even though odds tend to overestimate the true risk.[64,65] If the outcome is considered rare (<10% prevalent in the study population), the risk ratio and odds ratio approximate each other. However, the margin of overestimation increases as the outcome becomes more common. This is problematic given that the prevalence of preterm birth is >10% among pregnant women living with HIV. This challenge may be addressed by simply choosing a different model to directly estimate risk, like log-binomial regression, or estimating weighted cumulative incidence curves using survival analysis.

Another way to estimate effects using observational data is to emulate a target trial.[67–70] In the most literal sense, a target trial is a hypothetical RCT that we would wish to conduct under ideal circumstances. Thinking about studies in this way offers a useful heuristic to clarify the study design and the corresponding claims of effect we wish to make.[67] When emulating a target

trial with observational data, the target trial is the RCT we would design with the variables contained within the observational dataset we intend on using, assuming of course that the dataset contains sufficient information on confounders to approximate baseline randomization.[68,69] If the emulation is successful, the results would be comparable to results from the target trial, had it been conducted. To be clear, the emulated target trial will essentially be a pragmatic trial, when treatments are compared under conditions in which they would usually be applied, to study subjects more representative of real world patient population.[113] This is in contrast to a more classically designed RCT with stringent inclusion and exclusion criteria which produce results with high internal validity but may lack in its ability to generalize results to the patient population for whom the results were intended.[113] Further description of pragmatic trials are available elsewhere.[67,121,122]

The value in analyzing observational data using the target trial lens lies in the ability to approach causal inference explicitly, as opposed to the implicit (and perhaps informal) attempts at causal analyses using observational data.[69] Doing so offers investigators a structured process for assessing observational studies and their ability to make causal claims.[67,69] In this paper, we briefly discuss the specific mechanisms of designing a target trial; then we go through a worked example of how to emulate a target trial using observational data from the Women and Infant Transmission Study to estimate the effect of ART on preterm birth; [1,3] and finally we discuss the quality of the emulated trial as per our worked example and lessons learned.

5.2 Methods

The specific components of a target trial and how to emulate it using observational data are described in detail by Hernán and Robins.[67] We describe here how to appropriately define the eligibility criteria, determine the mechanics of treatment assignment and emulate randomization. The eligibility criteria for an emulated target trial analysis should be identical to that of the target

trial.[67,69] Further, eligibility cannot be determined by events occurring after baseline for the same reason that in a true randomized trial setting, it would be impossible to include women into a study by conditioning on future events. In an emulated trial setting, treatments are consistent with baseline characteristics and remain constant throughout the study to approach the intent-to-treat principle. Since randomization is a key component absent from observational study designs, we can either assume that exposure is unconditional, or we can assume that exposure is conditional on baseline characteristics and control for baseline covariates in the analysis to achieve comparability between treatment groups.[67,69] For this study, we outlined the target trial components alongside our emulated target trial protocol in Table 5.1.

The aim of the target trial (and by extension the emulated target trial) is to estimate the comparative causal effect of a newly initiated HIV treatment regimen at 20 weeks gestation on preterm birth among HIV-positive pregnant women. Women are eligible for randomization in the target trial if they are HIV-positive; their pregnancies survive to 20 weeks; are ART-naïve prior to 20 weeks of pregnancy; and have no history of alcohol or drug abuse, AIDS-defining illness, Type I/II diabetes; genetic or fetal abnormalities, or are taking medication that is contraindicated with study exposures. Women are randomized at 20 weeks to either PI cART, NNRTI cART, ZDV monotherapy and No Therapy.

5.3 Study Population

The emulated target trial analysis uses data from WITS collected between 1990 and 2005. The WITS was a prospective, interval cohort of pregnant women living with HIV. Women were allowed to enroll at any time during pregnancy and were followed until the end of pregnancy. Exposure and covariate data were collected at each study visit using standardized study questionnaires, as well as through retrospective medical record abstraction. There was no lost to follow up or right-censoring. Further details about this cohort are available elsewhere.[1,2] In our

emulated target trial, we included women from WITS who were unexposed to ART at conception and were pregnant with a single fetus surviving to 20 weeks.

In contrast to the target trial, inclusion to the emulated trial was not dependent on prior alcohol abuse, drug abuse, history of diabetes, or history of hypertension. These will be accounted for in the analysis. If a woman contributed multiple pregnancies to the WITS cohort, we included only her first pregnancy into the analysis population. Women not exposed to one of the four treatment strategies at their first exposure were also excluded. Additionally, women were eligible for "treatment assignment" if they enrolled into WITS between 18 and 22 weeks gestation (Figure 1). This differs from the described target trial since both eligibility assessment and randomization occur at 20 weeks, however we consider the window of 18 to 22 weeks to be narrow enough to still be reasonably consistent with the target trial protocol. Further detail on exposure definition is in a following section.

5.4 Outcome Classification

Pregnancies ending in live birth (hereafter referred to as "birth" or "delivery") as a result of spontaneous labor onset between 20 weeks and 36 6/7 weeks were considered preterm. A secondary outcome was considered combining preterm births and all stillbirths. This secondary outcome was of interest because both preterm birth and stillbirth can be caused by ART exposure.[49,79] Very preterm (20 – 32 weeks) and extremely preterm (20 – 28 weeks) were not considered in this analysis due to the rare occurrence of these events in our data.

5.5 Exposure and Confounder Classification

Exposures considered in this analysis are from drug classes prescribed at the time of data collection, namely PIs; nucleoside reverse transcriptase inhibitors (NRTIs); and non-nucleoside reverse transcriptase inhibitors (NNRTIs). Exposure categories for the emulated target trial were defined as no therapy, Zidovudine (ZDV) monotherapy; PI-based cART; and NNRTI-based

cART. If ZDV monotherapy was prescribed concurrently with cART, the more suppressive, combination therapy was prioritized for exposure categorization.[22] Women starting on any combination not falling into these categories were excluded from the analysis. Women in the emulated target trial analysis were prescribed an exposure corresponding to the time of enrollment (occurring between 18 and 22 weeks gestation). We were unable to confirm if women were ART-naïve at conception due to incomplete data on treatment history and timing of HIV diagnosis, but we assumed that if women were unexposed at enrollment or data from medical record abstraction indicated that women initiated treatment in pregnancy shortly prior to enrollment, they were likely unexposed as conception.

Baseline information about each pregnancy was collected at the WITS enrollment visit through standardized enrollment questionnaires. Race/ethnicity was classified into four racial categories (American Indian or Alaskan Native, Asian or Pacific Islander, Black, and White) and two ethnic categories (Hispanic origin, and Not of Hispanic origin) as per the 1996 U.S. Census Bureau conventions. Racial and ethnic categories were combined into a single race/ethnicity category in the WITS as White, Black, Hispanic and other (including Native American/Alaskan Native and Asian/Pacific Islander). BMI was calculated using weight and height at enrollment visit and categorized into the following standardized categories from the study period:[123] <19.8, 19.8 to 26.0; and >26.0. The following covariates were all coded dichotomously as yes/no: pre-pregnancy diabetes; pre-pregnancy hypertension; pre-pregnancy diagnosis of an AIDS-defining illness; and reported use of cigarettes, marijuana, crack/cocaine, heroin or alcohol at the WITS enrollment visit. WITS follow-up spanned two decades with evolving HIV treatment guidelines; treatment era was categorized as follows: 1990 to 1994 when there was no standard HIV treatment; 1994 to 1996 when ZDV monotherapy was the standard of care in pregnancy; and

1996 to 2005 when suppressive, combination therapy was introduced and scaled up (also known as highly active ART or HAART). Baseline viral load measurements, CD4 count and maternal age were recorded at enrollment into the WITS and modeled as restricted quadratic splines with four equal knots.[100]

5.6 Statistical Methods

Maternal characteristics were summarized, with stratification according to ART regimen at baseline. We emulated randomization of the target trial in two ways: first by assuming that treatment assignment was unconditional, and second that treatment assignment was conditional on measured baseline covariates. For this second approach, we used the minimally sufficient set of baseline covariates based on a causal diagram to estimate causal effects (Appendix Figure A.2). We calculated the crude risk of preterm birth by regimen and used modified Poisson models with robust variance estimators to estimate risk ratios (RR) and 95% confidence intervals.[74] Risk ratios were estimated for the effect of baseline ART on preterm birth, comparing each exposure group to PI cART as the reference.

We conducted an identical analysis to examine how sensitive our emulated target trial estimates were to widening the enrollment window from 18 to 22 weeks to 12 to 28 weeks. This sensitivity analysis included women enrolling at any time in the second trimester of pregnancy and was the prescribed treatment was based on that recorded at the time of enrollment.

5.7 Missing Data

Missing values were dealt with using multiple imputation (MI).[75,124] While we had complete exposure and outcome data, there were several baseline covariates we wished to include in our multivariate model which had missing values. We included variables in the MI models that were used in the analytic models for the main effect, as well as auxiliary variables to improve the overall fit of the imputation model.[76] We then performed multiple imputation by

chained equations, generating 30 imputed datasets for each analysis. We then ran the analysis multivariate model on each imputed dataset and pooled the resulting parameters using Rubin's Rule to derive the adjusted risk ratios and 95% confidence intervals.[124]

5.8 Results

Of the 3297 pregnancies in WITS, 2922 pregnancies were excluded for enrolling prior to 18 weeks or after 22 weeks; 18 pregnancies were excluded for being either multiple gestation, ending prior to 20 weeks or being from the same woman; 39 were excluded for preconceptional exposure to ART and 58 were excluded for starting on non-study therapy. The final analysis sample was 260 (Figure 5.2). Given our strict definition of eligibility as per the stated target trial, the resulting study sample is considerably smaller than the WITS cohort. As expected, the distribution of ART regimens corresponded to the treatment era of the pregnancy. Only pregnancies occurring after 1996 were subject to PI exposure since this regimen was unavailable prior to 1996, with very few pregnancies exposed to NNRTI cART overall. Values were missing for baseline viral load, BMI and history of diabetes and hypertension. Maternal characteristics stratified by exposure group and the corresponding proportion of missing values are presented in Table 5.2.

Based on our causal diagram, baseline covariates important to emulating randomization included race and ethnicity, maternal age, baseline BMI, history of substance use (including cigarettes, alcohol, marijuana and hard drugs), baseline viral load, pre-pregnancy AIDS-defining illness, study site and treatment era. From these covariates we identified a minimally sufficient adjustment set to achieve a parsimonious multivariate model, which included baseline viral load, pre-pregnancy AIDS-defining illness, treatment era, study site and maternal age. Study site was unavailable in our dataset. We imputed missing values of baseline viral load using the analytic

model variables and the following auxiliary variables: CD4 count, pre-pregnancy substance use in pregnancy, and race/ethnicity.

Assuming randomization in our emulated target trial to be unconditional, pregnancies started on No Therapy at baseline had a higher risk of preterm birth when compared to PI cART, though not significant [RR: 1.12, 95% CI (0.69, 1.81)] (Table 5.3). Comparing the other study exposures to PI cART yielded similarly insignificant results, though surprisingly, the risk ratio estimate for ZDV monotherapy suggested protection when compared to PI cART. We did not see materially different results when we assumed that randomization was conditional on baseline covariates. The multivariate analysis further suggested that women exposed to No Therapy would have a lower risk of preterm birth than those starting on PI cART [RR: 0.79; 95% CI: (0.42, 1.46)]. The sensitivity analysis included considerably more women by allowing enrollment throughout the second trimester. (N = 972) (Table D.1) Even so, the analysis did not offer results that differed from the primary emulated target trial, though we gained precision in our risk ratio estimates (Table D.2). Effect estimates examining the effect of ART on the secondary outcome (live still births combined with all stillbirths) were not materially different than that from the primary analysis and are not reported.

5.9 Discussion

In this analysis, we aimed to demonstrate how a target trial may be emulated using observational data. We expected the results of the emulated target trial to yield interpretable risk ratios showing PI cART preventing preterm birth when compared to no therapy and monotherapy, and that there would be no material difference between preterm birth risk between pregnancies starting on PI cART and NNRTI cART. After adjusting for identified baseline covariates present in the dataset, preterm birth risk for women starting pregnancy on PI cART at 20 weeks was higher when compared to all other exposures (though all risk ratios were

statistically insignificant.) This finding is contrary to what has been scientifically established in the ART literature; monotherapy is a decisively inferior treatment option compared to combination ART regimens, in relation to both preventing vertical HIV transmission and in preventing preterm birth.[37,125] One of the likely reasons for these surprising results is that our sample size was quite small to detect any meaningful effect between exposures given our strict target trial emulation eligibility criteria. Additionally, our dataset had a significant amount of missing data and measurement error, likely contributing to uncontrolled confounding at baseline and biasing our results.

Bias is essentially a missing data problem.[126] For this analysis, the nature of missingness may be divided into two groups. First, missing data in the form of measurement error is generally expected from an observational study setting. Measurement error may be introduced at virtually all stages of data collection and data entry, including but not limited to: identifying appropriate study participants; relying on participant recall for key data (including treatment exposures); ensuring the paper forms used for data collection are legible; following participants faithfully throughout follow-up; ensuring the reliable collection of all study variables; promoting consistency of study management throughout the observation time; entering the data into a database without error; etc.[127] The aforementioned list highlights the expected risks of an observational study which may be addressed, at least in part, through mitigation strategies throughout the study and analysis process. Even so, implementing a large, multi-site observational study like the WITS, spanning more than two decades is immensely challenging and we usually expect some presence of measurement error because of these challenges. It is particularly clear from the extent of missing baseline viral load data for example, that data quality was quite low, making the WITS a poor candidate for target trial emulation.

The second sort of missing data is a bit more specific to this analysis. In order to create a dataset for public use, the study team removed or altered key variables from the original dataset to ensure the confidentiality of the study participants. (The original dataset was destroyed as per NIH requirements.) This means that in our analysis, we were unable to account for variation by study site which would have been a baseline covariate to adjust for. We were also unable to consider other important baseline characteristics like parity and gravidity; and timing of exposure and gestational age in precise calendar time. Missingness of this kind is particularly problematic when pursing causal claims because we can no longer make safe assumptions regarding the absence of unmeasured confounding, nor can we assume – even in expectation – that the causal criteria are met.[128]

The hallmark strength of an RCT is that in principle, randomization distributes measured and *unmeasured* confounders between the exposure groups of interest such that a counterfactual population is approximated to facilitate a causal contrast. Recall that to usefully emulate a target trial, randomization is approached through controlling for important covariates at baseline and then preserving this "randomization" through an intention-to-treat analysis allowing for unbiased causal effect estimates.[67] We were ultimately unsuccessful because we were unable to emulate randomization reliably, thus yielding logically impossible results. Applying the target trial approach alone does not facilitate valid results.

The target trial approach can certainly be useful when the underlying observational dataset contains the necessary variables measured reasonably to simulate the conditions of a randomized controlled trial and has enough subjects across the exposures of interest. Future observational cohorts of ART exposures and their effect on adverse birth outcomes should consider this method to facilitate causal claims of risk. This approach would be particularly

feasible using large administrative datasets like Medicare or payor datasets, where achieving power would be less of barrier. Planning an observational cohort study knowing that the analysis method relies heavily on appropriate and complete measurement will set the stage for making powerful causal claims in the observational context. This is particularly relevant as new formulations of ART are coming on the market and vulnerable populations, like pregnant women, will need to be considered, not just for the prevention of vertical transmission, but also for the prevention of adverse birth outcomes.

5.10 Tables and Figures

Table 5.1. Study protocol summaries for the proposed target trial and the related emulated target trial to examine the causal effect of ART on preterm birth in the WITS dataset, 1990 – 2005.

Component	Target Trial	Emulated trial using WITS Observational Data
Aim	To estimate the comparative causal effect of a newly initiated HIV treatment regimen at 20 weeks gestation on preterm birth among HIV-positive pregnant women.	Same.
Eligibility	Women are eligible for randomization in the target trial if they are HIV-positive; their pregnancies survive to 20 weeks; are ART-naïve prior to 20 weeks of pregnancy; and have no history of alcohol or drug abuse, AIDS-defining illness, Type I/II diabetes; genetic or fetal abnormalities, or are taking medication that is contraindicated with study exposures.	Same as Target Trial except for the following: 1. Women are eligible if they enrolled into WITS between 18 and 22 weeks gestation. 2. Women should be unexposed to ART at conception 3. Inclusion will not be dependent on alcohol/drug use, diabetes or hypertension (these will be controlled for in analysis) 4. Pregnancies not exposed to one of the four treatment strategies as their first exposure will be excluded.
Treatment Strategies	PI cART, NNRTI cART, ZDV monotherapy and No Therapy	Same.
Treatment Assignment	Women are randomly assigned to one of four treatment strategies at 20 weeks gestation	Women are assigned the exposure recorded at their enrollment visit between 18 and 22 weeks. Randomization is emulated via adjustment for baseline covariates
Follow-up	Follow-up starts at treatment assignment at 20 weeks and ends at delivery, death or loss to follow-up. All enrolled pregnancies will be followed until delivery.	Follow-up starts at enrollment and ends at delivery. There is no right censoring, death or lost-to-follow up in these data.
Outcome	Outcome 1: spontaneous live births occurring prior to 37 weeks gestation Outcome 2: spontaneous live births occurring prior to 37 weeks gestation and all stillbirths	Same
Causal Contrast	Intention-to-treat effect, i.e., the effect of being assigned to No Therapy, ZDV Monotherapy or NNRTI cART compared to PI cART at 20 weeks	Same
Effect Estimate Interpretation	Causal	Causal
Statistical Analysis	Intention-to treat analysis.	Same

Figure 5.1. Timing of assessment of study eligibility (enrollment) and exposure assignment by study type.

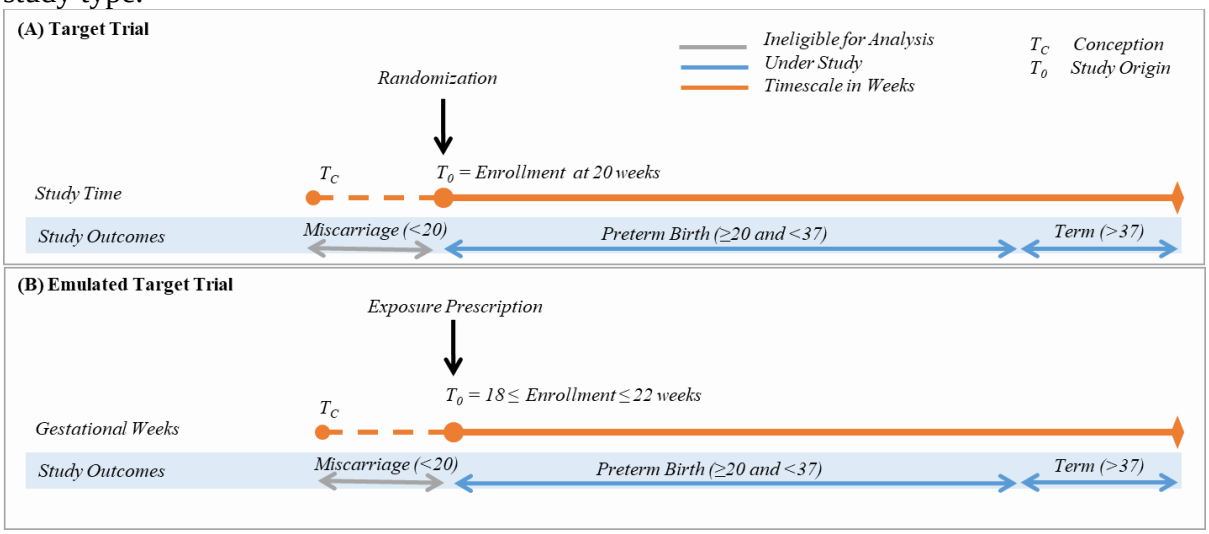


Figure 5.2. Consort Diagram for Emulated Target Trial.

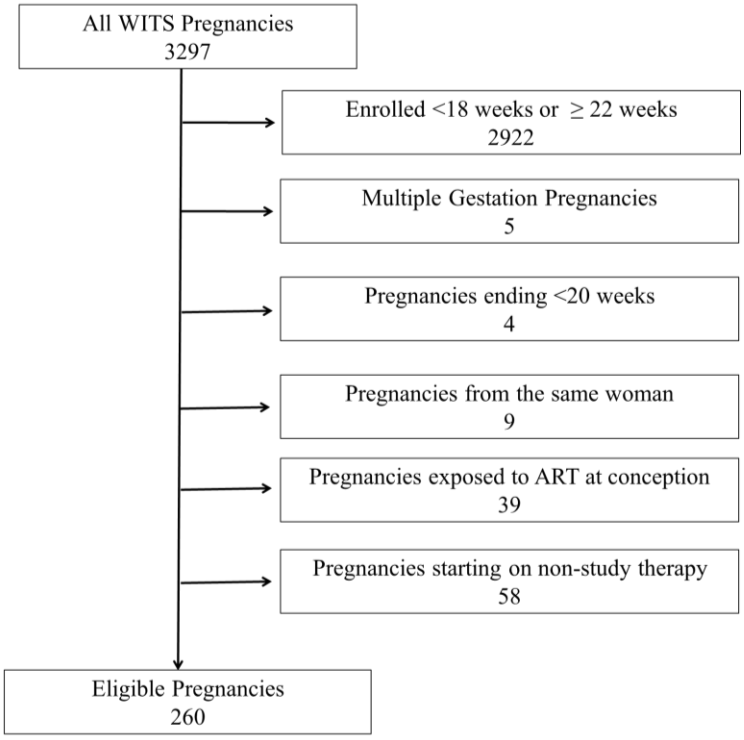


Table 5.2. Baseline and pregnancy characteristics of singleton pregnancies surviving past 20 weeks from eligible women enrolled in WITS between 1990 and 2005 (N = 260).

Characteristics	**PI cART** 102	**No Therapy** 111	**ZDV** 37	**NNRTI cART** 10
		Column Percentages (%)		
Race/Ethnicity				
White	6.9	9.9	10.8	10.0
Black	44.1	51.4	46.0	70.0
Hispanic/Latina	48.0	30.6	40.5	20.0
Other	0.0	0.9	0.0	0.0
Unknown	0.0	5.4	0.0	0.0
Missing	1.0	1.8	2.7	0.0
Maternal Age				
18-24	33.3	37.8	48.7	30.0
25-34	52.9	51.4	43.2	60.0
35-49	13.7	10.8	8.1	10.0
Treatment Era				
<1994	2.0	57.7	21.6	0.0
1994 to 1996	11.8	15.3	43.2	20.0
>1996	86.3	27.0	35.1	80.0
Baseline BMI*				
Less than 19.8	1.0	1.8	2.7	0.0
19.8 - 26.0	34.3	27.9	16.2	30.0
Greater than 26.0	33.3	31.5	18.9	50.0
Missing	0.8	38.7	62.2	20.0
History Substance Use				
Cigarettes	24.5	40.5	29.7	30.0
Missing	0.0	0.0	0.0	0.0
Alcohol	20.6	41.4	46.0	20.0
Missing	0.0	0.0	0.0	0.0
Marijuana	5.9	18.0	0.0	20.0
Missing	1.0	0.0	0.0	0.0
Hard Drugs	19.6	33.3	21.6	20.0
Missing	30.4	3.6	0.0	20.0
History Chronic Disease				
Hypertension	3.9	6.3	5.4	10.0
Missing	30.4	3.6	0.0	20.0
Diabetes	2.0	2.7	0.0	0.0
Missing	30.4	20.7	0.0	20.0

Characteristics	PI cART 102	No Therapy 111	ZDV 37	NNRTI cART 10
Gestational Age at WITS enrollment in Weeks				
Mean (IQR)	20 (19, 21)	20 (19, 21)	20 (19, 21)	20 (19, 21)
CD4 at Enrollment				
<200	10.8	7.2	10.8	10.0
201-349	20.6	12.6	13.5	10.0
350-500	21.6	15.3	5.4	20.0
>500	21.6	27.9	13.5	30.0
Missing	25.5	36.9	56.8	30.0
Viral Load at Enrollment (copies/ml3)				
<400**	20.6	1.8	8.1	30.0
401-10,000	26.5	12.6	10.8	20.0
>10,000	10.8	15.3	13.5	20.0
Missing	42.2	70.3	67.6	30.0
History of AIDS-defining Illness	4.9	6.3	16.2	0.0
Pregnancy Outcome				
Live Birth	98.0	91.0	97.3	100.0
Still Birth	2.0	2.7	0.0	0.0
Therapeutic Abortion	0.0	1.8	0.0	0.0
Spontaneous Abortion	0.0	4.5	2.7	0.0
HIV Transmitted to Child	2.9	12.6	5.4	10.0
Missing	3.9	9.9	8.1	0.0
Gestational Age at Delivery				
Term (≥37 weeks)	77.5	74.8	81.0	90.0
Preterm (20 - 37 weeks)	22.6	25.2	18.9	10.0
Very Preterm (20 - 32 weeks)	3.9	11.7	2.7	10.0
Extremely Preterm (20 - 28 weeks)	0.0	9.9	2.7	0.0
Mode of Delivery				
Elective C-Section	25.5	11.7	5.4	10.0
Non-Elective C-Section	12.8	7.2	18.9	30.0
Assisted Vaginal Delivery	4.9	3.6	0.0	20.0
Spontaneous Vaginal Delivery	44.1	61.3	62.2	30.0
Vaginal Delivery (assisted status unknown)	0.0	1.8	0.0	0.0
C-Section (elective status unknown)	3.9	3.6	5.4	10.0
Missing	8.8	10.8	8.1	0.0

*BMI categorization is based on standards relevant at the time of data collection.[37]

*Viral suppression was defined as (<400 copies/ml3) at the time of study enrollment; the current definition is (<50 copies/ml3)

Table 5.3. Risks and Risk Ratios of preterm birth by baseline exposure among women eligible for the emulated target trial analysis in the WITS cohort with 95% confidence intervals, assuming that randomization is unconditional (Analysis 1) and that randomization is conditional on baseline covariates (Analysis 2).

			Analysis 1		Analysis 2**	
	Number of Events	Risk (%)	RR	95% CI*	RR	95% CI
Emulated Trial	*N = 260*					
PI cART	23	22.6	1.		1.	
No Therapy	28	25.2	1.12	(0.69, 1.81)	0.79	(0.42, 1.46)
ZDV Monotherapy	7	18.9	0.84	(0.39, 1.79)	0.74	(0.32, 1.72)
NNRTI cART	1	10.0	0.44	(0.07, 2.9)	0.50	(0.07, 3.4)

**Model adjusted for the following minimally sufficient baseline covariates to emulate baseline randomization: baseline viral load, pre-pregnancy AIDS diagnosis, treatment era and maternal age.

CHAPTER 6: DISCUSSION

6.1 Overview

Understanding the safety and efficacy of HIV treatment in pregnant populations is of urgent importance given the potential adverse effects on pregnancy and birth. Reconciling the confusion around the true effect of ART on preterm birth continues to be a challenge since some studies suggest differential harm, particularly implicating PI cART [43,48,81–83] while other studies show no difference in protection among suppressive regimens. [57,84,85] Reconciling these conflicting findings.[43,48,81–83] Having as many valid analysis approaches as possible in the toolkit is essential to making sense of possible harms and benefits of ART, particularly during pregnancy. Our study reexamined the effect of PI cART on preterm birth by illustrating the use of alternative epidemiologic methods.

For the first aim, we departed from the use of binary regression methods since they do not easily accommodate time-varying exposure. Reanalyzing the WITS data to estimate preterm birth risk utilizing survival methods, we hypothesized that the effect of PI ART on preterm birth would not be different than that among pregnancies exposed to other combination therapies. For the second aim, we demonstrated the use of an analysis approach perhaps more appropriately using binary methods to understand the effect of ART on preterm birth to make causal claims. We hypothesized that the effect of PI ART on preterm birth derived from the emulated target trial would not be different than that among pregnancies exposed to other combination therapies, but would be protective compared to monotherapy.

6.2 Contributions

First, the use of survival methods is certainly not novel in pregnancy studies nor is it new in HIV studies, however we wanted to demonstrate its value in the context of HIV and pregnancy since this approach has not yet been fully embraced as a prioritized analysis method when the effect of treatment is studied in HIV populations. By estimating risks and risk differences, we were able to offer interpretable results that could more easily be applied to clinical practice and policy. Further, our analysis showed that by using survival methods, we were able to examine risk of preterm birth over the entirety of gestation, and not just at the conventional cut-points. This method also accounts for the realities of observational cohorts like exposures changing during pregnancy, late entry and competing risks.

Second, ours is the first study to use an emulated target trial approach to facilitate causal claims reexamining the effect of ART on preterm birth. It is difficult to study pregnancy in a randomized context given the ethical concerns of exposing a fetus to exposures with unknown effects. Because of the this, emulating a target trial offers a compelling way to approach an RCT environment while utilizing data from an observational cohort. Employing this method does not dispense with the usual limitations of observational studies; however it does mitigate the analysis-induced pitfalls often found in the literature like selection bias and immortal person-time bias. The value of analyzing observational data using the target trial lens lies in the ability to approach causal inference explicitly.

6.3 Limitations

There were several limitations to note when considering the conclusions of this study in their entirety, primarily having to do with the quality of the data we used for both aims. First, we used data from an observational cohort that started enrolling women almost 40 years ago and ended 15 years ago. HIV treatments and populations at risk have evolved significantly since

then, so the actual findings derived from our study are not clinically relevant in the present. Even though PIs are still utilized in sub-Saharan Africa, they are mostly considered as second line treatment, after virologic failure of first line ART.[129] Additionally, the introduction of integrase inhibitors in 2012 and the scaling up of this drug class since then has shifted focus away from the ART regimens that were prominent in the 1990s and early 2000s.[130] Even so, we hope that by demonstrating the use of appropriate methods in the HIV and pregnancy context, we can derive better inferences on the effect of new ART drug classes in pregnancy.

A related limitation is that the findings from this study may not be entirely generalizable to the U.S. population of pregnant women living with HIV today. The use of drugs and alcohol in pregnancy has significantly reduced among women living with HIV compared to the time at which these data were collected.[131] Additionally, people are risk of HIV are more frequently tested and linked to care given the expansion of universal test and treat programs in the United States.[132] This means that a higher proportion of women would have initiated ART prior to conception. In contrast, our analysis is limited to pregnancies who started treatment after enrollment. We were unable to reliably identify women who initiated preconceptionally due to the limitations associated with exposure timing.

Third, the WITS data are vulnerable to missing data and measurement error given that the study spanned more than 20 years of observation. Study protocols were updated, and several study sites were included and removed over this period. Data quality concerns are generally expected from an observational study setting since error may be introduced at virtually all stages of data collection and data entry, including but not limited to: identifying appropriate study participants; relying on participant recall for key data (including treatment exposures); ensuring the paper forms used for data collection are legible; following participants faithfully throughout

follow-up; ensuring the reliable collection of all study variables; promoting consistency of study management throughout the observation time; entering the data into a database without error; etc. Even so, accounting for this level of variably is challenging when the analysis dataset lacks the sufficient variables to fully describe this sort of variation.

The study team removed or altered key variables from the original dataset to ensure the confidentiality of the study participants in order to create a dataset for public use. (The original dataset was destroyed as per NIH requirements.) This meant that in our analysis, we were unable to understand the distribution of the removed variables and whether they would have been identified as important confounders or modifiers. This was challenging because we can no longer (comfortably) make the conventional analysis assumptions regarding the absence of unmeasured confounding, nor can we assume – even in expectation – that any the causal criteria are met. This was particularly important in Aim 2 when we sought to emulate a target trial. Randomization was approached through controlling for important covariates at baseline and then preserving this "randomization" through an intent to treat analysis allowing for unbiased effect estimates. The absence or unreliability of key variables prevented us from deriving reasonable inferences. Our study demonstrated however that unless we have complete and reliable measurement of key covariates and confounders, emulating a target trial will be unsuccessful.

Finally, timing of exposure in pregnancy was subject to patient recall in combination with medical record abstraction. It was unclear from the dataset whether the exposure start and stop times were derived from medical records or patient recall; because of this, we had to assume that the data were accurate, irrespective of source. If dates were missing, assumptions were made to ensure that the field was not coded as missing. The data cleaning was conducted by the study team so were blinded to the decision-making that was not detailed in the study documentation.

Because these challenges are in part expected when analyzing observational data, mitigation strategies may be used in both the study implementation and analysis. That said, it was difficult for us to know to what extent such measures were successful in our analysis.

6.4 Conclusion

In conclusion, our study demonstrates the use of alternative methods through a worked example, for other researchers to consider when seeing to understand the relationship between ART and preterm birth. Our first aim examined the relationship of ART regimen on preterm birth using survival analysis to appropriately characterize changing exposure over the entire duration of pregnancy in the WITS population. Conventional analyses in the literature tend to categorize ART exposures as time-fixed in order to use binary analysis methods, and are often subject to selection bias or immortal time bias. Our study differed from these analyses by estimating risks and risk differences using the complement of the Kaplan-Meier estimator, accommodating time-varying exposure; and by prioritizing causal theory and substantive knowledge when considering covariate adjustment. Estimating risks and risk differences using survival analysis should be considered as an alternative to regression in an effort to harmonize methods across the field of study, particularly since the effect of new ART drugs in pregnancy are a priority.

For our second aim, we demonstrated how to emulate a target trial for facilitate making causal claims about the effect of ART on preterm birth using observational cohort data. In doing so, we also highlighted the qualities required in the observational dataset to conduct the analysis successfully. For successful trial emulation, the “time zero” needs to be correctly specified and the observational data set needs to have sufficient information on confounders to emulate randomization. Using the WITS was ultimately a poor choice to illustrate the merits of this approach. Even so, our second aim offers an alternative way to analyze observational data with

the trial is not feasible, particularly given the challenges in conducting RCTs with pregnant women.

Understanding the safety and efficacy of HIV treatment in pregnant populations is of urgent importance, given the constant introduction of new therapies. Designing cohort studies planning for the use of the analyses methods presented in this study will undoubtedly support appropriate and complete measurement of key variables, and will set the stage for making powerful causal claims in the observational context. Studying pregnant populations is challenging given the practical and ethical concerns. We urge researchers in this field to consider the methods presented in this study to facilitate causal claims of risk and to promote decisive public health policy and clinical action.

APPENDIX A: DIRECTED ACYCLIC GRAPHS

Figure A.1. Aim 1 Directed Acyclic Graph for the relationship between PI cART and preterm birth.

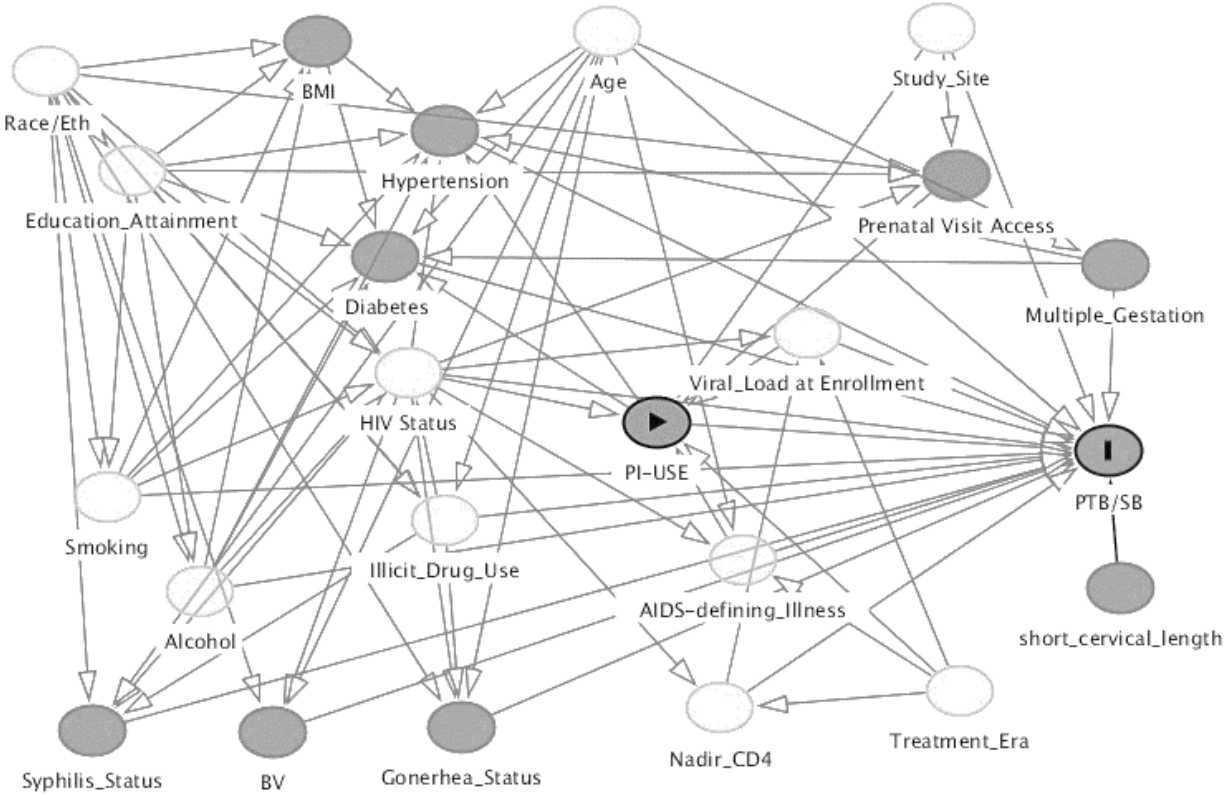
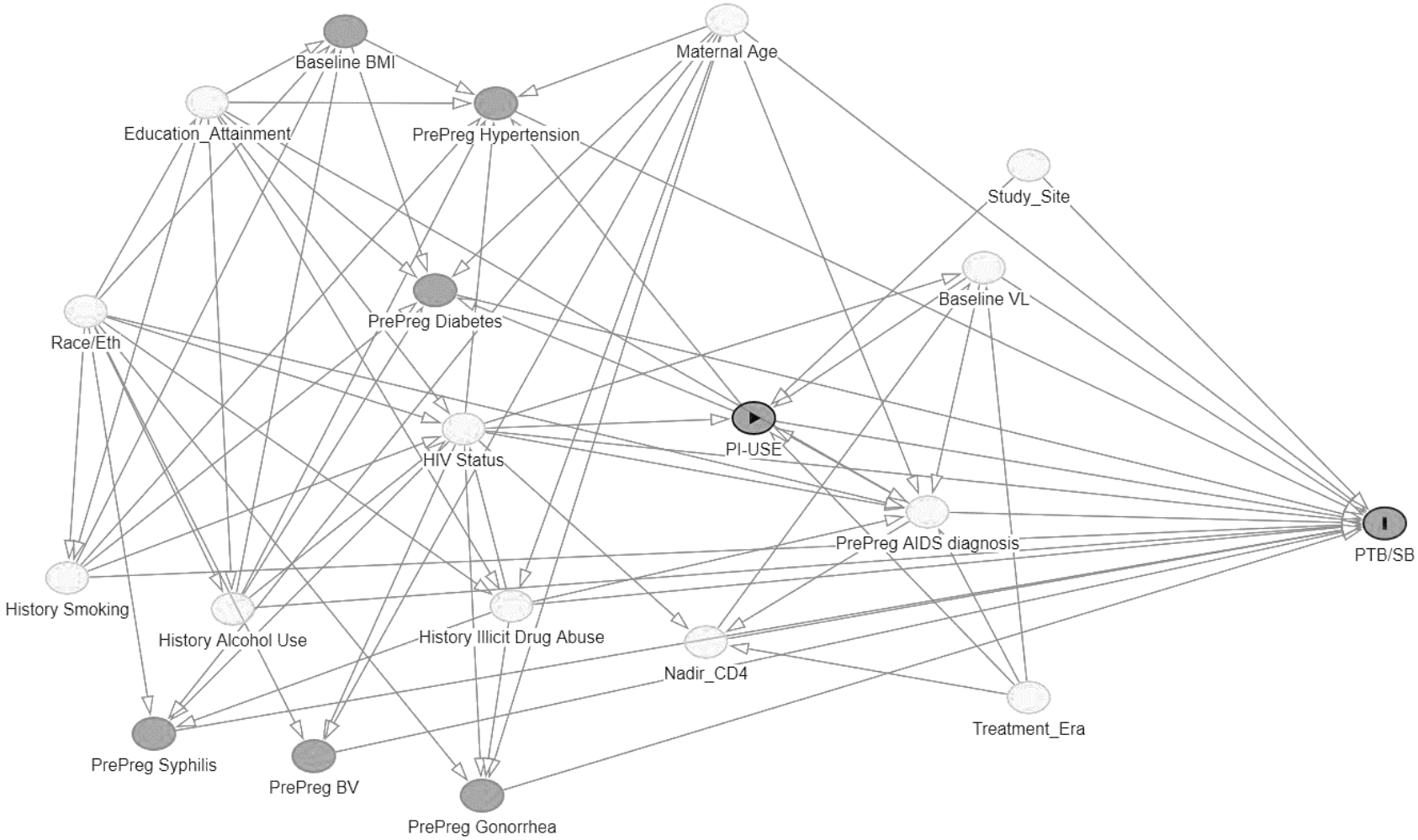


Figure A.2. Aim 2 Directed Acyclic Graph for the relationship between PI cART and preterm birth.



APPENDIX B: SUPPLEMENTAL DESCRIPTION OF EXPOSURES

Table B.1. Description of study exposures clarifying exposure definition and drugs comprising combination therapies.

Study Exposure Categorization	Description	Regimen *Name, Abbreviation (FDA Approval Year)*		
		Base Drugs		**Backbone Drugs****
Protease Inhibitor Combination ART (PI cART)	Combination of 3 or more drugs containing PIs and NRTIs (sometimes given concurrently with ZDV monotherapy as per pregnancy treatment recommendations)	Saquinovir, SQV (1995) Ritonovir, RTV (1996)* Lopinavir, LPV (2000) Atazanavir, ATZ (2003)	+	Zidovudine, ZDV (1987) Didanosine, ddT (1991) Lamivudine, 3TC (1995) Abacavir, ABC (1998) Tenofovir, TDF (2001) Emtricitabine, FTC (2003)
Non-nucleoside Reverse Transcriptase Inhibitor Combination ART (NNRTI cART)	Combination of 3 or more drugs containing NNRTIs and NRTIs (sometimes given concurrently with ZDV monotherapy as per pregnancy treatment recommendations)	Nevirapine, NVP (1996) Efavirenz, EFV (1998)		
Mixed Therapies *(effects not reported)*	Combination of 2 or more drugs including PIs and NNRTIs			
Zidovudine Monotherapy	ZDV only	Zidovudine, ZDV (1987)		
No Therapy	Unexposed to HIV treatment			

*In 2001, boosting with RTV was recommended for all PIs.

**All backbone drugs are nucleoside reverse transcriptase inhibitors (NRTIs)

APPENDIX C: AIM 1 - SUPPLEMENTAL TABLES AND FIGURES

Figure C.1. Histogram showing the distribution of gestational age at delivery among singleton pregnancies enrolled in WITS between 1996 and 2005 from women living with HIV (aged 15 to 45).

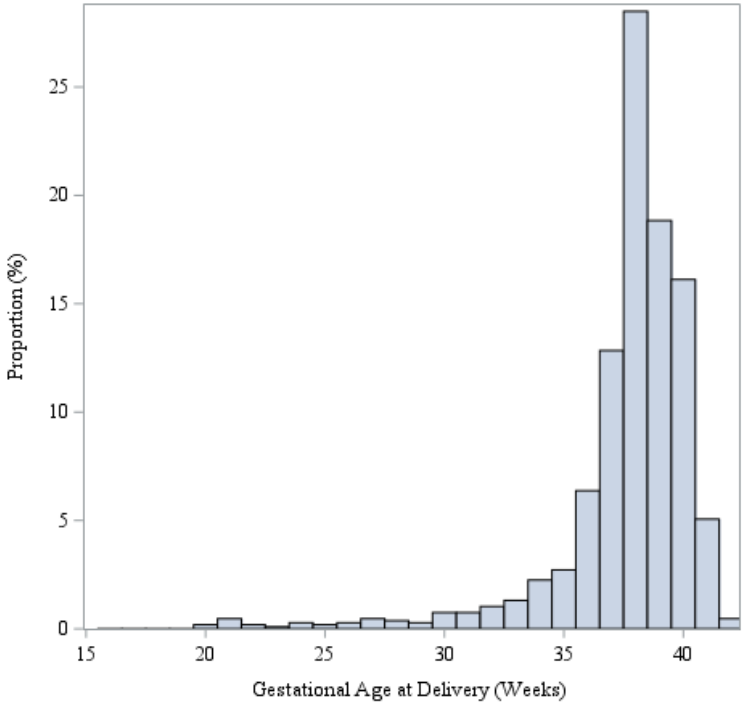


Figure C.2. Cumulative incidence curve for delivery among singleton pregnancies enrolled in WITS between 1996 and 2005 from women living with HIV (aged 15 to 45). Dashed lines appear at 20 and 37 weeks marking the preterm delivery period.

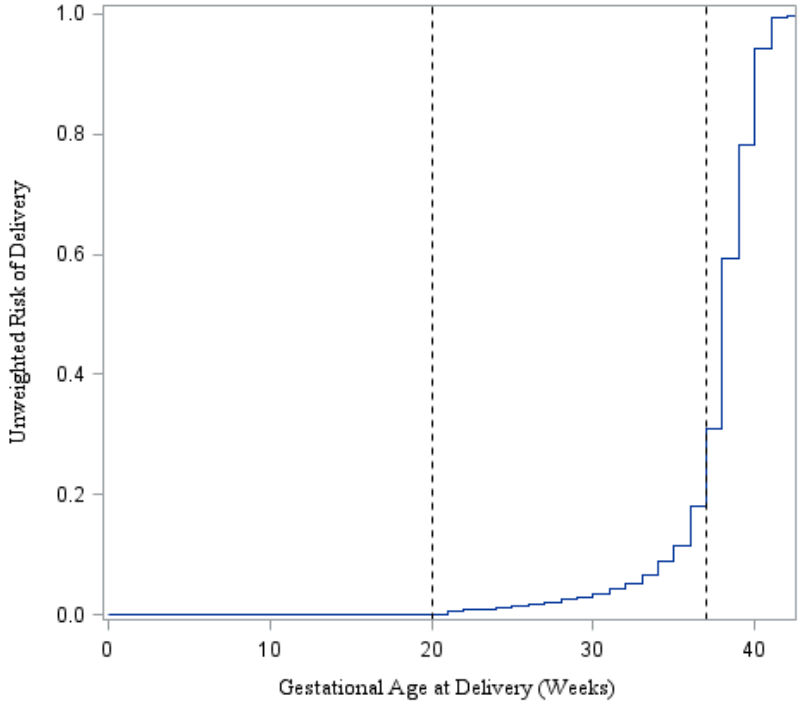


Table C.1. Weighted risks and risk differences for the effect of ART regimen on preterm delivery among women enrolled in WITS between 1996 and 2005 from women living with HIV (aged 15 to 45) with bootstrapped 95% confidence intervals (N = 932).

	Weighted*			
	Risk (%)	**95% CI**	**RD**	**95% CI**
Preterm (<37 Weeks)				
PI cART	0.15	(0.13, 0.18)	0	0
No Therapy	0.23	(0.20, 0.26)	-0.07	(-0.11, -0.03)
NNRTI ART	0.05	(0.00, 0.10)	0.10	(0.05, 0.16)
ZDV Monotherapy	0.16	(0.13, 0.19)	-0.01	(-0.04, 0.03)

*Weighted risks and risk differences accounted for baseline viral load and maternal age.

APPENDIX D: AIM 2 - SENSITIVITY ANALYSIS

Table D.1. Selected baseline and pregnancy characteristics of singleton pregnancies surviving past 20 weeks from eligible women enrolling between 12 and 28 weeks (2nd trimester) in wits between 1990 and 2005 (n = 972).

	PI cART	**No Therapy**	**ZDV**	**NNRTI cART**
Characteristics	292	491	146	43
		Column Percentages (%)		
Race/Ethnicity				
White	7.9	10.8	11.0	11.63
Black	44.2	49.9	43.8	69.77
Hispanic/Latina	44.9	33.2	39.7	16.28
Other	2.4	4.7	4.2	0.00
Unknown	1.7	1.4	1.4	2.33
Maternal Age				
18-24	36.6	37.3	43.2	39.5
25-34	52.4	53.0	45.9	44.2
35-49	11.0	9.8	11.0	16.3
Treatment Era				
<1994	1.7	54.6	18.5	0.0
1994 to 1996	9.9	21.2	43.8	16.3
>1996	88.4	24.2	37.7	83.7
Baseline BMI*				
Less than 19.8	3.8	2.4	1.4	4.7
19.8 - 26.0	32.2	38.1	33.6	30.2
Greater than 26.0	37.7	35.9	31.5	46.5
Missing	26.4	23.0	63.0	33.6
History Substance Use				
Cigarettes	29.5	45.2	37.7	30.2
Missing	0.0	0.0	0.0	0.0
Alcohol	25.7	47.7	38.4	23.3
Missing	0.0	0.0	0.0	0.0
Marijuana	9.3	17.9	14.4	14.0
Missing	1.7	0.0	0.0	0.0
Hard Drugs	17.8	39.7	28.8	23.3
Missing	0.0	0.0	0.0	0.0
History Chronic Disease				
Hypertension	4.5	7.5	11.0	4.7
Missing	27.7	3.3	1.4	32.6
Diabetes	2.1	1.8	2.7	0.0
Missing	27.4	19.6	3.4	32.6

Characteristics (continued)	PI cART	No Therapy	ZDV	NNRTI cART
	292	491	146	43
Gestational Age at WITS enrollment in Weeks				
Mean (IQR)	19 (16, 22)	22(18, 26)	18 (15, 23)	19 (16, 23)
CD4 at Enrollment				
<200	15.8	20.4	25.3	16.3
201-349	9.6	10.2	8.9	4.7
350-500	20.6	14.1	11.0	25.6
>500	20.9	18.1	21.2	18.6
Missing	33.2	37.3	33.6	34.9
Viral Load at Enrollment (copies/ml3)				
<400**	24.0	5.5	12.3	34.9
401-10,000	26.0	15.9	26.0	18.6
>10,000	20.2	13.6	15.1	18.6
Missing	29.8	65.0	46.6	27.9
History of AIDS-defining Illness	4.8	4.3	7.5	0.0
Pregnancy Outcome				
Live Birth	98.0	94.0	98.0	100.0
Still Birth	1.4	4.0	1.4	0.0
Therapeutic Abortion	0.3	0.4	0.0	0.0
Spontaneous Abortion	0.3	1.6	0.7	0.0
HIV Transmitted to Child	1.7	11.6	5.5	2.3
Missing	3.1	4.5	5.5	0.0
Gestational Age at Delivery				
Term (≥37 weeks)	81.2	77.8	80.1	90.7
Preterm (20 - 37 weeks)	18.8	22.2	19.9	9.3
Very Preterm (20 - 32 weeks)	3.4	6.9	6.2	4.7
Extremely Preterm (20 - 28 weeks)	1.7	4.28	4.11	0
Mode of Delivery				
Elective C-Section	24.32	8.76	8.9	20.93
Non-Elective C-Section	12.33	12.22	15.07	9.3
Assisted Vaginal Delivery	3.42	4.48	4.79	4.65
Spontaneous Vaginal Delivery	43.15	61.3	56.16	60.47
Vaginal Delivery (assisted status unknown)	1.37	3.05	2.05	0
C-Section (elective status unknown)	5.14	1.83	2.05	2.33
Missing	10.27	8.35	10.95	2.33

*BMI categorization is based on standards relevant at the time of data collection.[37]
*Viral suppression was defined as (<400 copies/ml3) at the time of study enrollment; the current definition is (<50 copies/ml3)

Table D.2. Sensitivity analysis identical to the primary analysis but allowing women to enroll throughout the second trimester of pregnancy. Analysis 1 assumes that randomization is unconditional (no model adjustment) and Analysis 2 assumes that randomization is conditional on baseline covariates.

			Analysis 1		Analysis 2**	
	Number of Events	Risk (%)	RR	95% CI*	RR	95% CI
Senstivity 1: Enrolling in 2nd Trimester	*N = 972*					
PI cART	55	18.8	1.		1.	
No Therapy	109	22.2	1.18	(0.88, 1.58)	0.94	(0.66, 1.35)
ZDV Monotherapy	29	19.9	1.06	(0.70, 1.58)	0.94	(0.62, 1.43)
NNRTI cART	4	9.3	0.49	(0.19, 1.29)	0.49	(0.19, 1.25)

**Model adjusted for the following minimally sufficient baseline covariates to emulate baseline randomization: baseline viral load, pre-pregnancy AIDS diagnosis, treatment era and maternal age.

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