Pre-exposure prophylaxis: primary prevention of HIV in at-risk populations

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BOSTON UNIVERSITY

SCHOOL OF MEDICINE

Thesis

PRE-EXPOSURE PROPHYLAXIS: PRIMARY PREVENTION OF HIV IN AT-RISK POPULATIONS

by

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PRE-EXPOSURE PROPHYLAXIS: PRIMARY PREVENTION OF HIV IN AT-RISK POPULATIONS

HOLLY MCHUGH

ABSTRACT

It is estimated that 50,000 individuals become newly infected with human immunodeficiency virus (HIV) every year in the United States. HIV is a lentivirus that is primarily spread through sexual contact. If left untreated, this viral infection can lead to decreased CD4+ T cells, increased susceptibility to opportunistic infections, and eventually progression to acquired immunodeficiency syndrome (AIDS) and death. HIV viral loads can be decreased to undetectable levels with the use of combination antiretroviral therapy (cART).

In 2012 the Food and Drug Administration approved cART therapy, Truvada (tenofovir/emtricitabine), for safe use as pre-exposure prophylaxis (PrEP). When detectable levels of drug are present in the blood stream of patients, there is up to a 92% relative risk reduction in HIV infection compared to placebo. The implementation of PrEP has the potential to decrease the incidence of new HIV infections in at-risk populations worldwide.

Because PrEP treatment is relatively new (2012), there are many barriers to administration to patients. Increased risky sexual behavior, known as risk compensation, is one of the concerns providers cite as a reason against prescribing PrEP. Most publications on PrEP have described randomized controlled trials that focused on safety, efficacy, and to a lesser extent, risk compensation behaviors. Now that Truvada is widely
available, researchers are starting to elucidate patients’ sexual habits while using PrEP in the outpatient settings. However, there is a need for more longitudinal research regarding the behaviors of individuals using PrEP, specifically to determine how often risk compensation occurs and under what conditions.

This study will initiate a PrEP clinic at Boston Medical Center and run a 3 year, open-label randomized controlled trial of eligible men who have sex with men (MSM) patients, who either start PrEP immediately or are delayed by one year. It is hypothesized that condom usage will decrease among immediate PrEP participants compared to the delayed participants. The study aims to determine if risk compensation occurs in these patients by following condom usage, development of sexually transmitted infections, number of sexual partners, and number/type of sexual encounter. Secondary outcomes will include measurements of medication adherence and number of HIV-seroconverters. These data will be collected through surveys and laboratory testing. The resulting information will help medical professionals better understand the risks and benefits of PrEP and also how to implement it most effectively in the fight to reduce the worldwide HIV burden.
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<table>
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<tr>
<td>AIDS</td>
<td>acquired immunodeficiency syndrome</td>
</tr>
<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
</tr>
<tr>
<td>BLT</td>
<td>bone marrow-liver-thymus</td>
</tr>
<tr>
<td>BMC</td>
<td>Boston Medical Center</td>
</tr>
<tr>
<td>cART</td>
<td>combination antiretroviral therapy</td>
</tr>
<tr>
<td>CDC</td>
<td>Centers for Disease Control</td>
</tr>
<tr>
<td>CMV</td>
<td>cytomegalovirus</td>
</tr>
<tr>
<td>CrCl</td>
<td>creatinine clearance</td>
</tr>
<tr>
<td>FDA</td>
<td>Food and Drug Administration</td>
</tr>
<tr>
<td>FTC</td>
<td>emtricitabine</td>
</tr>
<tr>
<td>HCW</td>
<td>healthcare workers</td>
</tr>
<tr>
<td>HIV</td>
<td>human immunodeficiency virus</td>
</tr>
<tr>
<td>HTLV</td>
<td>human T-cell leukemia virus</td>
</tr>
<tr>
<td>IN</td>
<td>integrase</td>
</tr>
<tr>
<td>IVDU</td>
<td>intravenous drug use</td>
</tr>
<tr>
<td>KS</td>
<td>Kaposi's sarcoma</td>
</tr>
<tr>
<td>LC-MS/MS</td>
<td>liquid chromatography–tandem mass spectrometry</td>
</tr>
<tr>
<td>LGBT</td>
<td>lesbian, gay, bisexual, transgender</td>
</tr>
<tr>
<td>LTR</td>
<td>long terminal repeat</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>MMWR</td>
<td>Morbidity and Mortality Weekly Report</td>
</tr>
<tr>
<td>MSM</td>
<td>men who have sex with men</td>
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<tr>
<td>NFAT</td>
<td>nuclear factor of activated T cells</td>
</tr>
<tr>
<td>NF-kB</td>
<td>nuclear factor kappa-light-chain-enhancer of activated B cells</td>
</tr>
<tr>
<td>NNRTI</td>
<td>non-nucleoside reverse transcriptase inhibitor</td>
</tr>
<tr>
<td>NRTI</td>
<td>nucleoside transcriptase inhibitor</td>
</tr>
<tr>
<td>NtRTI</td>
<td>nucleotide transcriptase inhibitor</td>
</tr>
<tr>
<td>OI</td>
<td>opportunistic infection</td>
</tr>
<tr>
<td>OLE</td>
<td>Open Label Extension</td>
</tr>
<tr>
<td>PBMC</td>
<td>peripheral blood mononuclear cell</td>
</tr>
<tr>
<td>PCP</td>
<td><em>Pneumocystis carinii</em> pneumonia</td>
</tr>
<tr>
<td>PI</td>
<td>principle investigator</td>
</tr>
<tr>
<td>PEP</td>
<td>post-exposure prophylaxis</td>
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<tr>
<td>PR</td>
<td>protease</td>
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<tr>
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<td>pre-exposure prophylaxis</td>
</tr>
<tr>
<td>RCT</td>
<td>randomized controlled trial</td>
</tr>
<tr>
<td>RT</td>
<td>reverse transcriptase</td>
</tr>
<tr>
<td>SHIV</td>
<td>simian human immune deficiency virus</td>
</tr>
<tr>
<td>SIV</td>
<td>simian immunodeficiency virus</td>
</tr>
<tr>
<td>STI</td>
<td>sexually transmitted infection</td>
</tr>
<tr>
<td>TDF</td>
<td>tenofovir disoproxil fumarate</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
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<tr>
<td>--------------</td>
<td>---------------------------------</td>
</tr>
<tr>
<td>UIAI</td>
<td>unprotected insertive anal intercourse</td>
</tr>
<tr>
<td>URAI</td>
<td>unprotected receptive anal intercourse</td>
</tr>
<tr>
<td>U.S.</td>
<td>United States</td>
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<tr>
<td>WHO</td>
<td>World Health Organization</td>
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CHAPTER 1 - INTRODUCTION

Background

Human immunodeficiency virus (HIV) is one of the major causes of morbidity and mortality in the world. According to the 2015 data from the World Health Organization (WHO), approximately 36.9 million people are living with HIV worldwide and there are nearly 2 million new infections every year. The disease killed an estimated 1.2 million people in 2014.¹

The greatest at-risk population for HIV infection in the United States (U.S.) is men who have sex with men (MSM), demonstrated by the fact that they make up only 2% of the total population, but carry the largest burden of new infection (63%). Antiretrovirals are now approved for primary prevention against acquiring HIV infections in this population. This is known as pre-exposure prophylaxis (PrEP). Truvada (see Table 2) is an antiretroviral combination of emtricitabine (FTC) and tenofovir disoproxil fumarate (TDF) that received Food and Drug Administration (FDA) approval in 2012 to be used for this indication. Because PrEP is still a relatively new concept, there remain many barriers to administration to patients. Increased risky sexual behavior, known as risk compensation, is one of the ongoing concerns that providers cite as a reason against prescribing PrEP to patients.²
**Statement of the Problem**

Most publications on PrEP have described randomized controlled trials (RCTs) that focused on adherence-based drug safety and efficacy, along with risk compensation behaviors. Now that Truvada is widely available, researchers are starting to study the sexual habits of patients on PrEP in the outpatient settings. In 2015, researchers at Kaiser Permanente published a prospective cohort study of 801 MSM in San Francisco, California to determine the incidence of Truvada associated risk compensation in the setting of a PrEP referral clinic. The study found that while sexually transmitted infection (STI) diagnoses increased and self-reported use of condoms decreased, there were no new HIV infections over the course of 388 person years. There is a need for more research like this to determine how the general MSM population will utilize PrEP and specifically to determine if risk compensation occurs, how often, and under what conditions over an extended period of time.

There are currently no PrEP clinics in the Boston area, despite Massachusetts having the 4th highest lesbian, gay, bisexual, transgender (LGBT) population density in the U.S. The Fenway Institute, which participated in the iPrEx study, offers access to PrEP in a primary care setting, but does not have a clinic specifically for PrEP patients. Opening a PrEP clinic would provide useful services to the LGBT community and has the potential to be a gateway for patients into the general healthcare system. It would also make following study patients much simpler compared to involving several different providers at multiple primary care locations.
Hypothesis

In the setting of an outpatient PrEP clinic, MSM taking Truvada will have a decrease in condom usage compared to MSM who have not yet started PrEP.

Objective and Specific Aims

The objective of this project is to initiate a PrEP clinic and to execute a study to determine if risk compensation occurs in the general population of MSM who are prescribed PrEP.

Specific aims:

1) To determine if statistically significant differences in sexual behavior occur between immediate PrEP initiators and delayed PrEP initiators. This will be measured in 2 ways:
   a) Surveys inquiring about condom usage, number of sexual partners, and number/types of sexual encounters
   b) Patient specimens to test for chlamydia, gonorrhea, and syphilis

2) To determine if statistically significant difference in sexual behavior can occur in patients before starting PrEP and after initiation of PrEP. This will be measured in 2 ways:
   a) Surveys inquiring about condom usage, development of STIs, sexual partner numbers, and sexual encounter numbers/types
   b) Patient specimens to test for chlamydia, gonorrhea, and syphilis

3) To determine if patients remain adherent to PrEP. This will be determined by checking blood samples to measure drug concentration levels.
4) To monitor the number of HIV-negative patients who become infected with HIV by performing regular HIV testing.
CHAPTER 2 – REVIEW OF THE LITERATURE

Overview of Research Topic

AIDs and the Discovery of HIV

During the early 1980s in the United States, acquired immunodeficiency syndrome (AIDS) was an unknown disease manifesting as life-threatening opportunistic infections (OIs) and cancer in homosexual men. However, it was not until several years of investigative research that the causative agent, HIV, was identified.

Michael Gottlieb, an astute Assistant Professor at University of California Los Angeles Medical Center, was the first to fit together some of the puzzle pieces. At the hospital, he identified a young homosexual male who had been admitted with fevers of unknown origin, weight loss, and a severely compromised immune system. Lab results confirmed that he was deficient in T lymphocytes, specifically CD4+ helper cells. The patient was discharged without a diagnosis and was re-admitted one week later with pneumonia. Lung tissue samples revealed that he was suffering from Pneumocystis carinii pneumonia (PCP), which more commonly causes disease in immunocompromised individuals. The patient passed away 2 months later.7

In the summer of 1981, Gottlieb et al. submitted an article to the Centers for Disease Control (CDC) Morbidity and Mortality Weekly Report (MMWR) where he identified 5 cases of PCP in young, sexually active homosexual men who were antibody positive for cytomegalovirus (CMV) and also had oral candidiasis. Three of the patients had “abnormal cellular-immune function.”8 These observations suggested that a common exposure may have led to cellular immunity dysfunction, therefore permitting the OIs to
develop. He theorized that the immunocompromised state could be due to CMV infection, but there was little data to support the idea.\(^8\)

Just a few weeks later, Friedman-Kien also published an article in MMWR describing the identification of 26 cases of Kaposi’s sarcoma (KS) in young, homosexual men located in California and New York which had developed within the previous 2.5 years. This was highly unusual, as most cases of KS until that time were in elderly men of Mediterranean descent. All patients that were tested for CMV were positive, and it was proposed that CMV may be playing an oncogenic role in the development of KS. This was also the first publication to associate a sexual preference (MSM) with cases of KS.\(^9\)

This article caught the nation’s attention\(^7\) and the CDC began to take action.

The CDC formed a surveillance task force in 1981 to collect data over a five-month period looking for cases of this unknown disease,\(^10\) defined as: “biopsy-proven KS among persons <60 years of age, or biopsy- or culture-proven life-threatening or fatal OIs and no known underlying illness (e.g. cancer or immune deficiency disease), or history of immunosuppressive therapy.” The CDC reported 159 cases of KS, PCP, and other OIs. Many of the patients developed a constellation of symptoms that included fever, weight loss, diarrhea, and lymphadenopathy.\(^11\) The occurrence of overlapping diagnoses (KS and PCP) in homosexual men of similar age, race, and geographical location suggested a common etiology within the population. They determined that it was unlikely to be an artifact that these disease states were concentrated in the MSM population and they reviewed possible associations with CMV, intravenous drug use (IVDU), and inhaled amyl/isobutyl nitrates.
CMV was known to cause immune cell dysfunction and had been found in several KS biopsies and also in a cell line derived from one KS patient.\textsuperscript{12,13} IVDU was of interest because a recent study had found several cases of PCP in drug users who were immunocompromised without an identifiable underlying disease.\textsuperscript{14} It has also been proposed that inhaled amyl/isobutyl nitrates may play a role because of their increased use as recreational drugs in the homosexual community.\textsuperscript{11}

At the end of 1981 Gottlieb et al. did further studies of this unknown disease found in 4 previously healthy, young men who developed PCP and mucosal candidiasis. All patients were leukopenic and suffered specifically from lymphopenia. They looked at the T-lymphocytes in peripheral blood mononuclear cells (PBMCs) by testing for proliferation after stimulation with mitogen (phytohemagglutinin) and antigen (\textit{Candida} and streptokinase-streptodornase), which occurs primarily in CD4+ helper T cells. They discovered decreased proliferation when compared to healthy controls, suggesting that there was a deficiency in either the function or number of CD4+ helper T cells.\textsuperscript{15}

Immunofluorescence was used to distinguish T lymphocytic subsets (total T cells, CD4+, CD8+) within the PBMCs. Compared to controls, the patients had a significantly lower number of absolute number of T cells (p<0.003), a significantly lower number of CD4+ helper T cells (p<0.0001), and a relatively higher percentage of CD8+ cytotoxic T cells.

All patients had acute or chronic infections with CMV, which can cause immunosuppression, including decreased CD4+ T cells. Therefore, the researchers proposed two theories: 1.) the disease was due to a new strain of CMV circulating through the homosexual population; 2.) the disease was caused by an unidentified
microorganism, drug, or toxin that creates an immunocompromised state, leading to OIs.\textsuperscript{15} Although the study did not identify the causative agent, they determined that CD4+ T cells were targeted by this deadly disease process.

By September 1982, the CDC had identified 583 cases of the disease they were now calling AIDS. It was defined as “at least moderately predictive of a defect in cell-mediated immunity, occurring in a person with no known cause for diminished resistance to [OIs].” The case mortality rate was >41%.\textsuperscript{16} Due to the high mortality, the CDC declared this epidemic a serious public health problem.

HIV was identified in 1983 in the oncogenic retrovirus lab of Luc Montagnier. Barré-Sinoussi et al. studied a cervical lymph node removed from an at-risk homosexual male, with >50 sexual partners/year and a history of STIs, who presented to the clinic with lymphadenopathy and general malaise.\textsuperscript{17} Enlarged lymph nodes had been identified as part of the initial clinical presentation that preceded AIDS.\textsuperscript{18} T lymphocytes from the biopsy were cultured and supernatants were assayed for RNA virus genome synthesis via \[^{3}H\] uridine incorporation and reverse transcriptase (RT) activity via the addition of template primers for cDNA (poly(A)-dT\textsubscript{12-18}) and Mg\textsuperscript{2+}. RT activity was detected at day 15 of cell culture and remained detectable for 15 days before decreasing concomitantly with the decline of T lymphocytes. Budding of the virus from the plasma membrane was visualized using electron microscopy. They also demonstrated that it was possible to infect healthy T lymphocyte donor cells with supernatant from the initial culture. When compared to the known human T-cell leukemia virus-1 (HTLV-1), this virus had an internal antigen (p25) similar to HTLV-1 antigen p24 and antibodies from the patient’s
serum reacted with proteins from HTLV-1. Similarities were not compared via sequencing.\textsuperscript{19} This patient was showing clinical signs of an early infection and was still immunocompetent when the biopsy was taken making it more likely that this newly identified virus, HTLV-III, was the causative agent rather than an OI.

With the use of this culture method, other researchers\textsuperscript{20} were able to identify HTLV-III in patients with pre-AIDS (lymphadenopathy or leukopenia with decreased helper T cells) and AIDS (severe immunodeficiency with decreased helper T cells accompanied by several OIs), which supported the idea that this virus was the causative agent of AIDS. In 1986, it was proposed by the International Committee on the Taxonomy of Viruses that the AIDS retrovirus be designated as HIV due to the fact that several labs had coined different terms for the same virus.\textsuperscript{21} While Françoise Barré-Sinoussi was unaware of the relevance at the time, she and Luc Montagnier would eventually win the Noble Prize in Physiology in 2008\textsuperscript{22} for their discovery of the HIV virus.

\textit{Epidemiology of HIV/AIDS}

HIV is one of the major causes of morbidity and mortality in the world. According to the 2015 data from the WHO, more than 36 million people are living with HIV worldwide and there are nearly 2 million new infections every year. The disease killed approximately 1.2 million people in 2014. The infection burden is highest in Sub-Saharan Africa, accounting for 70\% of cases of HIV globally.\textsuperscript{1}

In 2012 there were 1.2 million HIV-positive persons in the United States and it is estimated that every year 50,000 Americans become newly infected. While they account
for 12% of the population, African Americans make up the majority of new HIV infections (44%). Hispanic/Latinos account for 17% of the population and make up 23% of the new HIV infections. Caucasians, who make up the majority of the U.S. population (>70%), account for 27% of new HIV infections.23

While MSM only represent about 2% of the US population, they carry the greatest burden of new infection. In 2014, 67% of the estimated 47,500 new cases of HIV were in MSM (followed by heterosexual intercourse (24%) and IVDU (6%)). Based on results from the National HIV Behavioral Surveillance System from 2010, 19% of MSM who were screened for HIV were found to be infected with the virus. HIV prevalence by race within the MSM population was highest among African Americans at 28%, followed by Hispanic/Latinos at 18%, and Caucasians at 16%. When the incidence of transmission is considered by race, Caucasian and African American MSM account for nearly half of the estimated new HIV infections in the U.S.24

HIV can be transmitted through a variety of body fluids from infected persons. These include blood, pre-seminal fluid, semen, rectal fluids, vaginal fluids, and breast milk. The most common mode of transmission is through sexual contact with an HIV-positive individual without the use of condoms, PrEP, or combination antiretroviral therapy (cART). This occurs in the MSM population, heterosexual population, serodiscordant couples, and sex workers.25

Anal intercourse is the highest-risk sexual behavior and receptive positioning is associated with a higher risk of infection than penetrative positioning. The second highest-risk sexual behavior is vaginal intercourse, with females being at greater risk of
infection compared to males. HIV can also be transmitted via sharing needles, vertical transmission from mother to child (perinatally and via breast milk), and accidental needle sticks in health care workers (HCW). In the past, it was common for HIV to be transmitted by blood transfusion, blood products, or organ/tissue transplant, but this is rare in developed countries due to the rigorous screening processes that are now in place.  

HIV incidence has been reduced since it was first identified through screening of blood products, rigorous infection control in healthcare settings, public health education, and use of cART. However, HIV incidence remains high in the United States and worldwide. There is a need for additional methods of HIV prevention to further reduce the rate of new HIV infections. PrEP is a new chemotherapeutic approach that may augment existing strategies.
The human immunodeficiency virion is approximately 145nm in diameter. The outermost layer is an envelope comprised of a phospholipid bilayer derived from the host cell in which transmembrane viral attachment proteins and fusion proteins are inserted. Within the envelope is a cone-shaped nucleocapsid which contains the genome consisting of two copies of positive-sense, single-stranded RNA (See Figure 1).

The HIV genome is composed of 9 genes flanked by long terminal repeats (LTRs) (See Figure 2). The three major genes of the virus are gag, pol, and env. Gag encodes the matrix (p17), capsid (p24), and nucleocapsid (p7) proteins. Pol encodes enzymes that are
required for viral replication, integration, and maturation. These include integrase (IN) (p31), RT (p 51), and protease (PR) (p15).\textit{Env} encodes the viral envelope proteins gp120 and gp41. \textit{Tat} and \textit{rev} encode proteins with regulatory functions required for viral replication. \textit{Nef}, \textit{vif}, \textit{vpr}, and \textit{vpu} encode proteins that play accessory roles in the production of virus.\textsuperscript{28}

\textbf{Figure 3. Stages of the HIV life cycle and antiretroviral drug targets.}\textsuperscript{29} For viral entry into the host, the viral gp120 trimer must bind to the host cell CD4 surface molecule, which functions as the primary receptor for HIV.\textsuperscript{30} CD4 is expressed on T lymphocytes, monocytes, macrophages, and dendritic cells.\textsuperscript{31} The binding of gp120 to CD4 induces a conformational change in the viral glycoprotein allowing the virus to also bind to the co-receptor, which is required in addition to CD4 binding for viral entry into the cell.\textsuperscript{32} Most often this co-receptor is a chemokine receptor, either CXCR4 or CCR5, depending on the HIV variant. CCR5 is expressed on memory CD4+ T lymphocytes, dendritic cells, and macrophages, whereas CXCR4 is expressed on activated CD4+ T lymphocytes.\textsuperscript{31} After binding takes place, the gp41 trimer fusion
peptides are inserted into the plasma membrane of the host cell. This generates a conformation change in gp41 protein allowing for the formation of a six-helix bundle, creating a fusion pore between the viral envelope and the cellular membrane. The viral core enters the cellular cytoplasm where it undergoes “uncoating.” The capsid is shed while Vpr, IN, matrix, and viral RNA genome remain associated. As viral core “uncoating” continues, double-stranded cDNA is created from the viral RNA using viral RT. This becomes part of the pre-integration complex containing Vpr, IN, matrix and cDNA that then migrates into the nucleus where the cDNA is integrated into the host genome via IN to form provirus. This integration process occurs in two steps. First, integrase removes two nucleotides from the 3’ ends of the viral cDNA, exposing 3’-OH groups. Second, these nucleophiles attack the host DNA at the phosphodiesterase bonds of the DNA backbone, which results in the covalent integration of the 3’ ends of the viral DNA.

Once an infected cell becomes activated by antigen presentation, it expresses transcription factors, nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB), and nuclear factor of activated T cells (NFAT), which bind to the LTRs of the viral cDNA to initiate transcription of the provirus into mRNA. Viral mRNAs are produced as multiple, alternatively spliced species. Initial viral transcripts are heavily spliced and encode for proteins Tat and Rev. Tat enhances transcription of viral genes and stabilizes RNA transcripts for translation. As the amount of Rev protein increases, splicing of viral transcripts decreases, allowing for un-spliced viral mRNA genome to escape to the
cytoplasm. The full-length viral mRNA is translated to produce viral proteins and is packaged as the viral genome during virion assembly.\textsuperscript{38}

Virions are then released from the cell via budding from the plasma membrane. After release, Gag and Gag-Pol polypeptides are cleaved by the viral PR to generate the mature virus\textsuperscript{39} (See Figure 3). This process of replication from integrated provirus only occurs in activated T cells; however, HIV can also establish a latent reservoir in quiescent memory CD4+ T cells and macrophages.\textsuperscript{26}

\textit{Clinical Course of HIV Infection to AIDS}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure4.png}
\caption{Course of HIV infection in untreated patients.\textsuperscript{40}}
\end{figure}

HIV infection and progression to AIDS in the host is characterized by several phases (See Figure 4). The first phase begins after virus enters the host during the primary infection. During this acute phase, virus spreads from the point of entry, usually via mucosal membranes of the genital or anal tract or via blood, to the lymphoid system by invading follicular dendritic cells, allowing for systemic spread.
The acute phase often develops 3 to 6 weeks after the initial exposure to HIV. Some individuals will be asymptomatic, but 50-70% of individuals will have flu-like symptoms including fever, headache, sore throat, rash, and myalgia/arthralgia. During this phase, viral replication increases exponentially and HIV RNA becomes detectable in the serum. This is also when CD4+ T cells undergo a multifactorial decline through 1.) the cytopathic effects of virus; 2.) increased susceptibility to apoptosis; 3.) CD8+ T cells that recognize viral antigen in the context of MHC I.

In the weeks to months following the acute phase, patients enter the clinical latency phase. This occurs temporally with seroconversion which is when the adaptive immune system develops antibodies against HIV antigens. Viral symptoms resolve and the viremia decreases to a steady-state level. This phase lasts a median of 10 years, but eventually individuals experience depletion of CD4+ T cells, chronic inflammation, and ultimately advancement to AIDS. AIDS is defined by the CDC as a CD4+ count <200 cells/mm$^3$ and/or HIV-related OIs. Most patients usually die within 2 years of this diagnosis. Morbidity and mortality are due to diseases such as PCP, KS, tuberculosis, esophageal candidiasis, wasting syndrome, Cryptococcus neoformans infections, and Mycobacterium avium infection. Most patients follow this course unless viral replication is suppressed by cART.
HIV Treatment

There are many antiretroviral medications on the market today. They have been designed to interrupt the life cycle of HIV at different stages (See Table 1). Treatment for HIV is not curative. cART helps to control the viral load and improve CD4+ cell counts and prevent IOs.
Table 1.

<table>
<thead>
<tr>
<th>Type</th>
<th>Target</th>
<th>Mechanism of Action</th>
<th>Generic Name</th>
<th>Brand Name</th>
</tr>
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<tbody>
<tr>
<td>Entry Inhibitors</td>
<td>CCR5 co-receptor</td>
<td>Acts as an allosteric modulator of CCR5 that interferes with the binding of the viral gp120 to the target cell co-receptor CCR5</td>
<td>maraviroc</td>
<td>Selzentry</td>
</tr>
<tr>
<td>Fusion Inhibitors</td>
<td>Fusion protein gp41</td>
<td>Prevents the trimerization of gp41, which is required for fusion between the cell membrane and the virus particle</td>
<td>enfuvirtide</td>
<td>Fuzeon</td>
</tr>
<tr>
<td>Nucleoside Reverse Transcriptase Inhibitors (NRTIs)</td>
<td>Reverse Transcriptase</td>
<td>Acts as chain terminators because they lack the 3'-OH group, which is required for subsequent nucleotide incorporation during DNA polymerization</td>
<td>abacavir</td>
<td>Ziagen</td>
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<td></td>
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<td></td>
<td>didanosine</td>
<td>Videx</td>
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<td>emtricitabine</td>
<td>Emtriva</td>
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<td></td>
<td>lamivudine</td>
<td>Epivir</td>
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<td></td>
<td>stavudine</td>
<td>Zerit</td>
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<td></td>
<td>tenofovir disoproxil fumarate</td>
<td>Viread</td>
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<td></td>
<td>zidovudine</td>
<td>Retrovir</td>
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<tr>
<td>Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs)</td>
<td>Reverse Transcriptase</td>
<td>Binds to reverse transcriptase, creating change that allosterically inhibits its ability to generate cDNA</td>
<td>delavirdine</td>
<td>Rescriptor</td>
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<td></td>
<td>efavirenz</td>
<td>Sustiva</td>
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<td>nevirapine</td>
<td>Viramune</td>
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<td></td>
<td>rilpivirine</td>
<td>Edurant</td>
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<tr>
<td>Integrase Strand Transfer Inhibitors</td>
<td>Integrase</td>
<td>Binds at the active site of the viral integrase and displaces the viral DNA 3'-OH group which acts upon the host DNA for integration</td>
<td>dolutegravir</td>
<td>Tivicay</td>
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<td></td>
<td></td>
<td></td>
<td>elvitegravir</td>
<td>Viteka</td>
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<td></td>
<td>raltegravir</td>
<td>Isentress</td>
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<tr>
<td>Protease Inhibitors (PIs)</td>
<td>Protease</td>
<td>Binds to protease, creating a conformational change that keeps the polymerase active site in an inactive orientation</td>
<td>atazanavir</td>
<td>Reyataz</td>
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<td></td>
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<td></td>
<td>darunavir</td>
<td>Prezista</td>
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<td>fosamprenavir</td>
<td>Lexiva</td>
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<td>indinavir</td>
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<td>tipranavir</td>
<td>Aptivus</td>
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<td>Generic Names</td>
<td>Brand Name</td>
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<tr>
<td>abacavir and lamivudine</td>
<td>Epzicom</td>
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<tr>
<td>abacavir, dolutegravir, and lamivudine</td>
<td>Triumeq</td>
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<tr>
<td>abacavir, lamivudine, and zidovudine</td>
<td>Trizivir</td>
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<tr>
<td>atazanavir and cobicistat</td>
<td>Evotaz</td>
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<tr>
<td>darunavir and cobicistat</td>
<td>Prezobix</td>
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<td>efavirenz, emtricitabine, and tenofovir disoproxil fumarate</td>
<td>Atripla</td>
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<tr>
<td>elvitegravir, cobicistat, emtricitabine, and tenofovir alafenamide fumarate</td>
<td>Genvoya</td>
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<tr>
<td>emtricitabine, rilpivirine, and tenofovir alafenamide</td>
<td>Odefsey</td>
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<tr>
<td>emtricitabine, rilpivirine, and tenofovir disoproxil fumarate</td>
<td>Complera</td>
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<td>emtricitabine and tenofovir disoproxil fumarate</td>
<td>Truvada</td>
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<tr>
<td>lamivudine and zidovudine</td>
<td>Combivir</td>
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<tr>
<td>lopinavir and ritonavir</td>
<td>Kaletra</td>
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There are two different inhibitors that can block the entry of the virus. The first is an allosteric modulator of CCR5 that interferes with the binding of the viral gp120 and the co-receptor CCR5 on the target cell. The second is a peptide that prevents the trimerization of gp41, which is required for fusion between the cell membrane and the virus envelope.

Many drugs have been designed to target reverse transcription. The two major classes are nucleoside/nucleotide and non-nucleoside reverse transcriptase inhibitors (NRTI/NtRTI and NNRTI respectively). NRTI and NtRTIs act as chain terminators because they lack the 3’-OH group required to incorporate the subsequent nucleotide during DNA polymerization. NNRTIs on the other hand, bind to RT, creating a conformational change that keeps the polymerase active site in an inactive orientation.
One drug has also been created to target cDNA integration. Integrase strand transfer inhibitor binds in the active site of the viral IN and displaces the viral DNA 3’-OH group (strand transfer nucleophile), which acts upon the host DNA for integration. Without this interaction, insertion of viral cDNA into the target cell genome is not possible.\textsuperscript{45}

The last part of the viral cycle that has been successfully targeted for drug development is the maturation of virus. PR cleaves HIV polypeptide precursors in the virion after release from the cell. Protease inhibitors prevent proteolysis by competitively binding to the PR active site.\textsuperscript{38}

RT does not have a proofreading mechanism, therefore errors in transcription that occur approximately every $3 \times 10^{-5}$ base pairs are not corrected.\textsuperscript{26} This, combined with the rapid production of virus and the errors introduced by host cell RNA polymerase during transcription of viral RNAs, allows for the development of extensive genetic variation within the virus population, i.e. quasispecies. NRTI, NNRTI, and PI are usually prescribed together to target multiple steps in the viral replication cycle (See Table 2). The degree of viral suppression afforded by cART greatly reduces viral evolution and the emergence of drug-resistant mutations. This is one of the reasons that adherence to medication is so vitally important in the treatment of HIV.\textsuperscript{46}

\textit{Treatment as Prevention}

Once pharmacologic treatments for HIV were identified, researchers started to think about the utility of antiretrovirals as part of a preventative approach for those at risk for HIV infection. There are several populations that could greatly benefit from
chemoprophylaxis, so researchers began conducting trials to determine if it was a viable option.

The first population of public health interest was HIV-positive pregnant women. If it were possible to reduce the transmission of HIV from mother to the fetus/newborn, it would decrease the most common way that young children contract the disease. In 1994, a randomized, placebo-controlled trial in the United States and France determined the safety and efficacy of the NRTI, zidovudine, in HIV-positive pregnant women after the first trimester. Four hundred and seventy-seven women entered the trial with 238 participants in the placebo arm and 239 in the treatment arm. They were between 14 to 34 weeks pregnant and currently untreated for their HIV with CD4+ counts >200 cell/μl. The treatment group took zidovudine (100mg orally, 5x daily) during pregnancy and through delivery (2mg/kg/hr IV during 1st hour, then 1mg/kg/hr IV until delivery). Postpartum, each infant received 6 weeks of zidovudine (2mg/kg orally, every 6 hours, x6 weeks) and was followed for 72 weeks. Infants were considered infected if their cultured PBMCs were positive for HIV. Thirteen infants in the treatment group became infected (8.3%, 95% CI, 3.9-12.8), while 40 infants in the placebo group became infected (25.5%, 95% CI, 18.4-32.5). Relative risk of perinatal HIV transmission was reduced by 67.5% (95% CI, 40.7-82.1) compared to the placebo group.47

The short term toxic effect of neonatal anemia was seen in 18% of newborns in the treatment group. It was most significant at 3 weeks of age, but resolved within 12 weeks after birth. There were no teratogenic effects on the newborns. Only minimal short
term toxic effects such as anemia, neutropenia, thrombocytopenia, abnormal serum electrolytes, and elevated liver function tests were observed in the treated mothers.\textsuperscript{47}

There are several possible reasons why zidovudine did not confer greater protection against vertical transmission of HIV. First, it is not clear when transmission occurs from mother to the fetus/newborn, therefore it is conceivable that some of the fetuses/newborns were infected before zidovudine treatment was initiated. It is also possible that some women were infected with HIV strains resistant to zidovudine. It may also be true that a single agent was insufficient to reduce the mother’s viral load. Lastly, non-compliance could have also played a role in the incomplete viral protection in the treatment group.\textsuperscript{47} This study showed that the use of antiretrovirals could prevent HIV transmissions to infants of HIV-positive mothers with minimal side effects.

A second population of interest was HCWs. These individuals are at increased risk for HIV infection due to accidental exposures, primarily through needle sticks. If taking antiretrovirals as post-exposure prophylaxis (PEP) could decrease transmission of HIV, it would make health care facilities a much safer place. PEP is similar to PrEP because it is a form of prophylaxis; however, this treatment is initiated after the HIV exposure instead of before. In 1997, Cardo et al. conducted a retrospective case-controlled study of 33 HIV-seroconverted case HCWs and 679 control HCWs from the United States, the United Kingdom, and France with percutaneous exposures to HIV-positive blood. For each patient, information was collected regarding age, sex, occupation, work location, treatment with PEP, and when PEP was started (if treated). Information regarding the source patient was also collected and included stage of HIV at
time of HCW’s exposure, use of cART at time of HCW’s exposure, and if death occurred within 2 months of HCW’s exposure. Additionally, information was collected regarding the incurred injury. This included type of device, the gauge of the needle, the procedure being performed and its urgency, use of gloves, time elapsed before exposure, presence/absence of visible blood, and severity of injury.

Nine case patients (47%) and 172 (64%) control patients took zidovudine after their exposure to HIV. Sixty-seven % of control and 89% of case patients had their first dose of PEP within 4 hours of exposure (p=0.28). Of these, 66% of controls and 44% of cases patients continued PEP for at least 4 weeks. The dosage of zidovudine varied, but was usually ≥1000mg/day (78% of controls, 75% of cases). Information about the range of doses was unavailable.

Using logistic-regression, they identified several risk factors for seroconversion which included deep injury, devices visibly contaminated with the source patient’s blood, devices that were initially placed in an artery/vein of the source patient, and if the source patient died within 2 months of the incident. Most importantly, they found that risk of infection as detected by seroconversion was reduced by 81% (95% CI, 48-94%) when the individual took zidovudine after exposure to the HIV virus. This study supplied enough supporting evidence for the CDC to update the guidelines for HCWs by recommending the use of PEP in HIV exposure management.

There was still one large population that had not been studied who could benefit greatly from chemoprophylaxis, and they were individuals at high risk for HIV infection due to lifestyle choices and behaviors such as serodiscordant couples, sex workers, MSM,
and IVDU. Mike Youle et al. were some of the first individuals who suggested research on the use of antiretrovirals as PrEP. While they believed in the pursuit of an HIV vaccine, there was worry about how long it might take to develop it and that it may not confer complete immunity. Therefore, alternative preventative strategies needed to be pursued in an attempt to decrease the number of new HIV infections. To them, the ideal drug for PrEP would concentrate to high levels in the blood in a short amount of time and would have a “high genetic barrier” to the development of drug resistance. They thought that availability of once-daily therapy such as TDF would be a good choice because of low rates of toxicity. It also exhibited post-exposure protection against simian immunodeficiency virus (SIV) in the macaque HIV model and post-exposure protection against HIV in HCWs.

Existing Research

PrEP in Animal Studies

Before any PrEP regiment could be tested in humans, the proof of concept had to be tested in animals first. Vaginal and rectal exposure studies (see below) performed in mice and non-human primates were performed to detect the efficacy of PrEP. FTC and TDF were the two drugs studied, separately and together, in pursuit of a suitable and efficacious option for PrEP. Both are NRTIs and are known as Truvada in the combined formulation.

Denton et al. looked at 13 adult humanized bone marrow-liver-thymus (BLT) mice to determine if the use of FTC/TDF given prophylactically could prevent vaginal transmission of HIV. Treatment mice (n=8) were given FTC/TDF (3.5mg and 5.2mg)
intraperitoneally at 48 hours and 3 hours before all animals (control (n=6) and treatment) were vaginally inoculated with HIV-1<sub>JR-CSF</sub> (170 ng of p24) under anesthetization with sodium pentobarbital. The treatment group was then given intraperitoneal FTC/TDF once daily for 4 days after the HIV inoculation. Drug concentrations were not monitored. Every 2 weeks, the plasma viral load was determined by real-time PCR of viral DNA, plasma HIV antigen levels were measured using p24 ELISAs, and levels of human CD4+ and CD8+ cells were counted using flow cytometry. After inoculation, mice were evaluated daily with these tests for a minimum of 50 days.<sup>53</sup>

Eighty-eight percent (7 out of 8) of control BLT mice that were vaginally inoculated with HIV-1 had detectable viral load (minimum detectable copies = 5) as early as 2 weeks post-inoculation. The appearance of HIV in the plasma was temporal with a decline in peripheral CD4+ cells. Additionally, 100% of mice treated with FTC/TDF did not show detectable HIV.<sup>53</sup> Although the total sample size was small (14 mice), the study demonstrated the early promise for PrEP.

Subbarao et al. used 12 male Rhesus macaques in a study to determine if the use of TDF given prophylactically could prevent rectal transmission of simian human immune deficiency virus (SHIV), which is a model for HIV in humans. Four animals received oral TDF (22 mg/kg body weight) daily, 4 received oral TDF (22 mg/kg body weight) once weekly, and 4 received no TDF. Treatment animals were administered prophylaxis 2 hours before each rectal inoculation. Under anesthesia, all animals were inoculated with SHIV<sub>SF162P3</sub> (3.8x10<sup>5</sup> viral RNA copies) once weekly for 14 weeks or until the animals became infected with SHIV. HIV RNA in plasma was detected using
real-time PCR, IgM/IgG antibodies against HIV were detected using Western blots, and plasma levels of TDF were determined using high-performance liquid chromatography. The animals were followed for 37 weeks.

The control animals became infected after a median of 1.5 weeks, while animals prophylactically treated with oral TDF, either daily or weekly, had a delay in infection of a median of 7 and 6 weeks, respectively; this however was not statistically significant (p=0.315) compared to the control macaques. Plasma levels of TDF were checked in treatment animals during various weeks (undefined). TDF reached its peak concentration (633ng/mL) at about 2 hours after administration in both the daily and weekly oral treatments with TDF. Viral load was checked weekly and peaked within 1-2 weeks after the initial detection of plasma HIV RNA, in both treated and untreated animals. The HIV antibody response developed 3-7 weeks after initial detection of plasma HIV RNA in all groups. The infected animals did not express the K65R mutation, which can occur with monotherapy treatment of HIV with TDF.54 While the sample size was small and the difference between groups was not statistically significant, the study showed that TDF could delay HIV infection, but not prevent it.

Garcia-Lerma et al. went a few steps further than Subbarao et al. They performed a similar once-weekly rectal SHIV virus challenge (7.63x10^5 RNA copies) under anesthesia in adult Rhesus macaques for 14 weeks. The first group of animals (n=6) were treated with daily subcutaneous FTC daily (20mg/kg). The second group (n=6) was treated with daily subcutaneous FTC and daily oral TDF (22mg/kg). Group 3 (n=6) was treated with daily subcutaneous FTC and TDF (22mg/kg). Group 4 (n=6) was treated
with intermittent subcutaneous FTC and TDF. All animals were treated 2 hours before inoculation. Group 4 was only treated 2 hours before and 24 hours post inoculation each week. Eighteen control Rhesus macaques did not receive any treatment. HIV RNA in plasma was detected weekly using real-time RT-PCR, IgM/IgG antibodies against HIV were detected weekly using Western blots, and plasma drug levels were determined using liquid chromatography–tandem mass spectrometry (LC-MS/MS). The animals were followed for a total of 6.5 months.55

The untreated controls became infected with SHIV after a median of 2 exposures. Four out of 6 animals receiving daily FTC alone became infected, which represents a 3.8-fold decrease in infection risk (p=0.02) compared to control animals. Two of 6 animals in group 2 (daily subcutaneous FTC/oral TDF) became infected, with a reduction in infection risk of 7.8-fold compared to controls (p=0.008). Group 3 (daily subcutaneous FTC/TDF) and group 4 (weekly subcutaneous FTC/TDF) had complete protection from SHIV infection (p=0.00005). Of the animals infected while on treatment, 4 had wild-type virus and two had the resistance mutation M184V against FTC.55 This study showed that combination FTC/TDF was a better candidate for PrEP than simply FTC alone because it conferred greater protection against repeated SHIV challenges.

These studies on the effects of PrEP in preventing SHIV infection after vaginal and rectal exposures supported the idea that antiretrovirals may be effective for primary prevention of HIV infections. The proof of concept experiments in animals opened the door for randomized control trials in humans.
FDA Approval of PrEP

Many PrEP clinical trials have been completed in several high-risk populations such as MSM, heterosexual serodiscordant couples, heterosexual men and women, and IVDU. Two of those studies led to approval of PrEP by the FDA.

In 2010, Grant et al. published the first study of PrEP completed in humans, called iPrEx, which had MSM participants from Peru, Ecuador, South Africa, Brazil, Thailand, and United States. It was a phase 3, randomized, double-blinded, placebo-controlled trial aimed to evaluate the safety and efficacy of once-daily oral Truvada (200mg FTC, 300mg TDF) compared to placebo. Criteria for inclusion were >18 years old, male sex at birth, HIV-seronegative status, and evidence of high risk for acquisition of HIV. High risk behaviors meant that in the last 6 months, participant had anal sex with ≥4 male partners, an STI diagnosis, a history of transactional sex, or condomless anal sex with HIV-positive or unknown status partner. Two thousand four hundred and ninety-nine patients were randomized to daily oral use of TDF/FTC (n=1251) or placebo (n=1248). They did not include substance abuse history as part of the high-risk analysis.

Every 4 weeks, patients had follow-up to report pill adherence, undergo rapid testing for HIV antibodies, and received education importance of Truvada compliance and risk-reduction strategies. Additionally, to determine adherence, pills were counted and a new 30-day supply was dispensed. Every 12 weeks they were interviewed regarding any engagement in high risk behaviors (see above). Every 24 weeks, participants were given physical exams and screened for STIs (syphilis, herpes simplex virus-2, gonorrhea, and chlamydia).
Grant et al. also collected drug concentration data on a subgroup of the individuals taking Truvada to see if it correlated with a protective effect. They did this by looking at the presence of Truvada in the serum and by testing PBMCs for active intracellular metabolites of the drugs.\textsuperscript{3}

The cohort was followed for a total of 3324 person-years. The median length was 1.2 years/participant. Sexual practices regarding receptive intercourse and use of condoms remained similar in the two groups (p=0.97). The most common lab abnormality was elevated creatinine (26 in the treatment group), which resolved in all participants after discontinuation of the study drug. The most common side effects included moderate nausea (22 events) and unintentional weight loss (34 events).\textsuperscript{3}

By the end of the study, 100 patients had become infected with HIV; 36 out of 1251 in the FTC/TDF group and 64 out of 1248 in the placebo group, which is a 44\% reduction in HIV incidence (95\% CI, 15-63, p=0.005). This result would have been strengthened by drug level data for all individuals taking PrEP instead of just a subset. Patients are not always great historians and may be forgetful or dishonest about reporting adherence. If this drug data were known, it is possible that reduction in HIV incidence would have been greater when compared to placebo because people without consistent drug levels could have been eliminated from the analysis. Along these lines, Grant et al. discovered that in the subpopulation tested for blood drug levels, adherent patients with detectable drug levels correlated with successful chemoprophylaxis and a relative reduction in HIV infection of 92\% (95\% CI, 40 to 99, p<0.001) compared to those without detectable drug levels. This incomplete reduction may be due to the fact that
protective drug levels may vary depending on the type of body tissue exposure (penile vs. anal).³

The second study was the Partners PrEP trial published in 2012. It was a phase 3 randomized, double-blinded, placebo-controlled trial of 4758 serodiscordant heterosexual couples in Uganda and Kenya to evaluate the safety and efficacy of once-daily PrEP regimens (300mg TDF or 300mg/200mgTDF-FTC). Criteria for inclusion for the seronegative partner was ≥18 years of age, HIV-negative status, ≥6 episodes of vaginal intercourse with HIV-positive study partner in the last 3 months, adequate renal/hepatic/hematologic function, and chronic active HBV-negative. They excluded women who were pregnant or breast feeding. Criteria for inclusion for the seropositive partner were ≥18 years of age, HIV-positive status by ELISA, ≥6 episodes of vaginal intercourse with HIV-negative study partner in the last 3 months, a CD4+ cell count ≥250 cells/μl, and did not meet AIDS criteria.⁴

The seronegative partner was randomized to daily oral TDF (n=1584), Truvada (n=1579), or placebo (n=1584). HIV-negative patients had follow-up every 4 weeks when they received 2 rapid HIV antibody tests, and were educated on medication compliance and risk reduction strategies. Patients were also interviewed about their engagement in sexual risk-taking, including topics such as total number of intercourse acts, frequency of unprotected sex, and intercourse with non-primary partners. Unused pills were collected to check adherence and new 30-day supplies were dispensed. Serum analysis was performed every 3 months. Every 12 months an STI evaluation was completed with a physical exam and serum/urine testing.⁴
Seropositive partners received follow-up at 3-month intervals to obtain primary care services for their HIV. Every 6 months their CD4+ counts were checked and cART was initiated if they became eligible. Participants became eligible for antiretroviral treatment if CD4+ counts decreased below 350 cells/µl in Kenya and below 250 cells/µl in Uganda. Every 12 months an STI evaluation was completed with a physical exam and serum/urine testing.

The study was meant to follow patients for 35 weeks, but was ended early because it was evident that there was significant protection against HIV in the active PrEP arms of the study. Risky sexual behavior such as sex without a condom and sex outside of the study partnership remained similar between participants in all 3 arms of the study. The most significant lab abnormality was neutropenia seen in the Truvada arm of the study (p<0.001), but not in the TDF or placebo arms. The most common side effects of both PrEP regimens were gastrointestinal and fatigue.

Eighty two patients became infected during follow-up; 17 in the TDF group (n=1584), 13 in the FTC/TDF group (n=1579), and 53 in the placebo (n=1584). Compared to placebo, there was a 67% reduction in HIV acquisition with TDF (95% CI, 44-81%, p<0.001) and a 75% reduction with FTC/TDF (95% CI, 55-87%, p<0.001). The protection afforded by TDF alone versus FTC/TDF was not statistically significant (p=0.23). As with the Grant et al. study, it would have been beneficial to have drug level data on all the seronegative participants as patients can be dishonest with pill counts. Baeten et al. did look at a subset of participants to compare drug levels in those who seroconverted as compared with those that were still HIV-negative. Adherent patients
with detectable drug levels corresponded with a relative risk reduction of 86% in TDF arm (95% CI, 0.05-0.43, p<0.001) and 90% in the FTC/TDF arm (95% CI, 0.02-0.44, p=0.002).\textsuperscript{4}

These two studies in MSM and serodiscordant heterosexual couples showed that TDF/FTC significantly reduced the risk of HIV infection in high-risk seronegative individuals and helped pave the way for FDA approval of PrEP in 2012.\textsuperscript{58} The CDC has since published clinical practice recommendations that guide providers through a comprehensive HIV/STI prevention plan which includes administration of PrEP, education on condom usage, risk reduction counseling, HIV/STI testing, and STI treatment.\textsuperscript{59}

Truvada is a once daily, fixed-dose combination of TDF (200mg) and FTC (300mg), that is currently approved by the FDA for use as PrEP in MSM, heterosexual men/women, and IVDU who meet the recommended CDC criteria. TDF alone may be considered as an alternative for heterosexual men/women and IVDU as it has been proven effective in trials in these specific populations. The CDC does not recommend less than daily dosing of PrEP.\textsuperscript{59}

\textit{Benefits of Access to PrEP}

There are many potential benefits for patients who have access to PrEP in addition to decreased HIV transmission rates. PrEP users have reported enhanced sexual pleasure between partners\textsuperscript{60} by improving aspects such as bonding, intimacy, and spontaneity. PrEP also decreases fear of HIV infection, which has a large affective value during intercourse.\textsuperscript{61,62}
For some, PrEP brings empowerment because individuals can dictate their own HIV protection rather than relying on a sexual partner to use condoms, take cART appropriately, or honestly disclose their HIV status.\textsuperscript{60} This also correlates with increased pleasure and decreased fear. Additional benefits include decreased anxiety, depression, and sexual compulsivity for those taking PrEP.\textsuperscript{62}

\textit{Barriers to PrEP Access}

In 2013, 1175 active physician providers of the Infectious Disease Society of America in the U.S. and Canada were electronically surveyed to assess attitudes, readiness, and current practices of PrEP. Five-hundred and seventy-three physicians (48\%) responded to the survey and included an equal proportion of members employed by hospital/clinics, private practices, universities/medical schools, and VAs/military. The majority (74\%) of the respondents supported the use of PrEP, while 14\% were unsure, and 12\% did not support it. Even though the support was strong, only 9\% had actually prescribed PrEP, 43\% had not prescribed it, but would, 34\% didn’t think it was relevant to their practice, and 14\% would not prescribe it.\textsuperscript{2}

For those who would not prescribe PrEP, the most prevalent reason was apprehension concerning compliance and development of antiretroviral resistant viral strains (77\%). This was followed by uncertainties regarding cost and insurance issues (57\%), toxicity in healthy persons (53\%), and insufficient evidence for efficacy outside of clinical trials (53\%). There were several other barriers to prescribing PrEP. These included risk compensation, which is the idea that idea that risky behaviors will increase because of perceived decreased susceptibility to HIV. Several others had to do with
limitation of the provider’s knowledge regarding PrEP, limited clinic resources at the provider’s location, and personal philosophy.²

If it were possible to assuage health care providers of their perceived barriers to prescribing PrEP, it may help increase the use of PrEP as a treatment option for at-risk populations by the medical community. The next section will explore what is known about side effects, resistance, medication adherence, and risk compensation.

Side Effects of PrEP

In general, Truvada has been tolerated well by patients using it for PrEP. “Start-up syndrome,” which consists of mild gastrointestinal complaints in the first month of treatment, has been the most common side effect of PrEP. Some patients experience a combination of nausea, abdominal upset, loose stools, flatulence, and headache. These symptoms were experienced by 4% of the Truvada group in the Partners study and 13% of the Truvada group in iPrEx. These symptoms usually improved or completely resolved as treatment continued.⁴,³,₆

Truvada use in PrEP studies has only shown a small, but statistically significant reduction in creatinine clearance (CrCl) from baseline in the iPrEx study (-1.1ml/min) compared to the placebo group, which resolved when prophylaxis was discontinued.⁶³ According to the CDC guidelines, before initiation of the drug, patients require a creatinine clearance of 60 mg/dL and then receive regular monitoring of their kidney function once PrEP has been started.⁵⁹ This requirement makes monitoring this side effect straightforward.
Another documented side effect of TDF is a minor, but statistically significant, decrease in bone mineral density (1.1% net decrease at femoral neck (95% CI, 0.04-1.9%) and 0.8% net decline in total hip (95% CI, 0.3-1.13)) when compared to placebo. The decrease in bone density occurred around 6-9 months after starting TDF. However, patients were shown to have the same number of atraumatic fractures compared to untreated controls. Additionally, these decreases often stabilized or returned to normal as treatment continued. While bone density monitoring is not part of the CDC guidelines for PrEP, it can be performed if there is clinical suspicion or concern for bone loss.

To date, the side effects seen in PrEP clinical trials have been minimal, but the research periods have been relatively short with the longest follow-up being around 2 years. Data on long-term usage of antiretroviral drugs in healthy individuals are not yet available because the FDA approval for PrEP is so recent (2012), which means patients have not yet experienced extended exposure to the drugs. This is important information to obtain because the population that will benefit the greatest from PrEP is young MSM. Statistically, they have the highest risk of acquiring HIV. If treated early, it means they could be treated with PrEP for decades at a time and be at risk for unknown long-term side effects.

*Development of Anti-Viral Resistant HIV During PrEP*

HIV replication has a mutation rate of approximately $3 \times 10^{-5}$ per nucleotide base per cycle of replication. This high mutation rate is due to errors made by both the viral RT during viral replication and the host-cell RNA polymerase II during viral transcription. Both enzymes lack a proofreading mechanism. When antiretrovirals are
administered, variants of the virus with mutations conferring resistance are selected for survival and proliferate. This is more likely to occur when the antiretrovirals are used as single agents or in suboptimal dosing.\textsuperscript{26}

Table 3.

<table>
<thead>
<tr>
<th>Study</th>
<th>Resistant Virus</th>
<th>Mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>iPrEx baseline</td>
<td>2 of 2 cases</td>
<td>Met184</td>
</tr>
<tr>
<td>iPrEx treatment</td>
<td>0 of 48 cases</td>
<td>none</td>
</tr>
<tr>
<td>iPrEx OLE baseline</td>
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<td>US MSM Safety Trial baseline</td>
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<td>US MSM Safety Trial treatment</td>
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<td>none</td>
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<tr>
<td>PROUD baseline</td>
<td>2 of 3 cases</td>
<td>Met 184</td>
</tr>
<tr>
<td>PROUD treatment</td>
<td>0 of 2 cases</td>
<td>none</td>
</tr>
</tbody>
</table>

*Baseline indicates infection occurred before or within 4 weeks of starting PrEP.

The majority of cases of resistance in PrEP clinical trials have been due to initiating treatment during an unrecognized acute HIV infection (See Table 3). These infections go undetected because the initial screening test is often for HIV antibodies, which are not detectable early in infection, lending to a false negative test result.\textsuperscript{3,4,5,6}

Resistance has also occurred in individuals after initiating PrEP, but the majority was not taking the medication as prescribed and did not have detectable levels of drug in their blood when the infection occurred. In the iPrEx study, 48 people in the treatment arm became infected.\textsuperscript{3} Only 2 of the 48 developed a Met184 mutation in the RT to TDF, and this occurred at baseline (see Table 3), which means these patients were acutely infected when they started the trial. The other 46 cases occurred while receiving PrEP and these individuals became infected with wild-type virus, most likely because they
were non-adherent to the medication. Without detectable PrEP drugs in the serum, there is no selective pressure for the population expansion of resistant HIV mutants, which explains why there were so few cases of resistant infections during the study in the non-compliant subjects.

Based on current studies, PrEP does not contribute significantly to the issue of drug-resistant virus because not as many individuals are using it as compared to cART. It is estimated that 25,000 individuals are taking PrEP in the United States. Outside of research trials, there has only been one reported case in the U.S. of a patient seroconverting while compliant on PrEP, however it is unclear if the individual was consistently using condoms. In 2012, approximately 450,000 people (37%) of the U.S. HIV-positive population were being treated with cART. Up to 22% of individuals eventually fail treatment due to the development of resistant HIV infections. Studies suggest that the major cause of treatment failure is due to lack of adherence because of toxicity, poor tolerability, or inconvenient dosing. Other less common causes include drug interactions, insufficient drug combinations, and low-level resistant HIV virus populations. This means that the majority of resistance to antiretrovirals develops within the larger HIV-positive population on cART compared to the HIV-negative population on PrEP.

The CDC guidelines recommend obtaining an HIV test before starting chemoprophylaxis and delaying the initiation of PrEP in patients who appear to have a viral prodrome. The currently recommended HIV test is a combination immune assay that detects HIV-1 and HIV-2 antibodies and HIV-1 p24 antigen in patient’s blood. If the
samples are positive, they undergo further testing for re-confirmation and to differentiate the HIV antibodies. This screening process is the best way to prevent inadvertently prescribing PrEP to HIV-positive patients, preventing the primary source of resistant HIV virus development in PrEP.

**Medication Adherence**

At this point in time, the CDC has only endorsed PrEP for daily oral use. Research has shown that as doses per week increase, so too does the protective effect. As part of predefined pharmacology sub-studies within the iPrEx, Anderson et al. looked at blood and PBMC samples to evaluate plasma and intracellular drug concentrations associated with decreased acquisition of HIV. They first evaluated data from the STRAND study, an open-label, crossover study of oral TDF in 24 HIV-negative MSM adults, each of whom received 2, 4, and 7 doses (dose unknown) of drug per week for 6 weeks under direct observation. Drug doses in serum and PBMCs were measured using LC-MS/MS. The median drug concentrations were 11 fmol/10⁶ PBMCs for 2 doses/week, 32 fmol/10⁶ PBMCs for 4 doses/week, and 42 fmol/10⁶ PBMCs for 7 doses/week. These data were used for comparison to the iPrEx study participants.

Forty-eight HIV-positive cases from the iPrEx study were each matched to 3 HIV-negative control participants (n=144). The HIV cases had 5.5-fold decreased frequency (p<0.001) in detection of intracellular PrEP at the time of HIV diagnosis compared to controls. Only 3 of the 48 HIV-positive cases had detectable TFV when they were initially diagnosed with HIV. The highest concentration identified was comparable to the 2 dose/week range in the STRAND trial and none were in the daily dosing range.
The low drug concentration may help explain why these individuals acquired HIV during the iPrEx study.\textsuperscript{72}

The most important finding of this study was the estimated drug concentration associated with HIV-1 acquisition. Oral TFV concentration of 16 fmol/10\textsuperscript{6} PBMCs is associated with a 90\% reduction in HIV acquisition relative to placebo. Oral TDV use confers 76\% protection if used 2x/week, 96\% protection if used 4x/week, and 99\% protection if used 7x/week.\textsuperscript{72}

These data indicate that medication adherence is necessary to obtain the protective effects of PrEP. These data are not necessarily generalizable to other populations as PrEP concentrates in rectal tissue 20-100 times higher than in vaginal tissues. Therefore women might require stricter medication adherence or higher doses to obtain the same protection.

The iPrEx Open Label Extension (OLE) cohort study recruited men and transgender women who have sex with men, who had previously participated in a PrEP clinical trial (ATN 082, iPrEx, and U.S. MSM Safety Study), to take Truvada for a 72-month period with the aim to measure PrEP uptake, adherence, and sexual practices that more resemble the outpatient setting. They followed 1603 HIV-negative people, of whom 1225 (76\%) had previously received PrEP (but not at the time of enrollment). One thousand two hundred and thirty (77\%) of the participants opted to receive PrEP. All individuals (including those who opted out of taking PrEP) had follow-up at weeks 4, 8, 12, 24, 36, 48, 60, and 72. At each visit patients were tested for HIV antibodies. Patients were tested for syphilis, herpes, and urethritis every 24 weeks or if symptomatic. Drug
concentrations were measured at 4, 8, or 12 weeks using dried blood spots and blood plasma. Seventy-three people became HIV-positive during the study. Thirteen cases occurred in the group not receiving PrEP (2.6 infections/100 person-years, 95% CI, 1.5-4.5) and 28 in the group receiving PrEP (1.8 infections per 100 person-years, 95% CI, 1.3-2.6). Drug concentrations of PrEP in dried blood spots were strongly associated with decreases in HIV incidence. No infections occurred when TDF concentrations were ≥700 fmol/punch which equates to the use of 4 to 7 doses/week. Concentrations this high were only identified in 33% of the patients taking PrEP. Concentrations of 611 fmol/punch (95% CI, 216-1006), which equates to the use of 2 to 3 doses/week was associated with a 90% risk reduction. This study provides data that strict medication adherence may not be necessary for good efficacy. More research on this topic is currently underway to determine if drugs may be effective in preventing infection at lower and less frequent dosages.

The iPrEx OLE study also looked at other aspects of PrEP such as patient demographics and self-reported adherence. They found that adherence to PrEP was better with increased age (30-39, 95% CI, 1.26-2.15, p=0.0002) (≥40, 95% CI, 2.39-4.53 p<0.0001), greater education (secondary, 95% CI, 1.59-2.48, p<0.0001) (post-secondary, 95% CI, 1.55-2.41, p<0.0001), unprotected receptive anal intercourse (URAI) (95% CI, 1.37-2.02, p<0.0001), and having an HIV-positive partner (95% CI, 1.05-1.99, p=0.03). Participants were surveyed about their compliance during the first 12 weeks of initiation of PrEP. Eighty-five percent (583 participants) reported taking PrEP within the
last 3 days before the visit. One hundred eleven (70%) of the 158 tested demonstrated clinically significant drug concentrations on dried blood spots. Dried blood spots were used as a novel biomarker for long-term drug adherence. In the iPrEx study, self-reported pill use was high in both the treatment and placebo arms (95% at week 12). In concordance, pill counts reflected the patient reporting (91% at week 52). However, when the 43 seronegative controls in the treatment arm were tested for serum drug levels, only 51% had detectable levels. Despite reporting compliance to the daily medication regiment, the serum drug levels were inconsistently low in comparison.

The iPrEx studies highlight some of the provider concerns about medication compliance. Even though the self-reported pill use was not proportional to the concentrations of drug in the blood, analysis of the data by Anderson et al. shows that perfect adherence is not necessary to gain the beneficial protection of PrEP. This idea of imperfect adherence is also supported by the data in the iPrEx OLE study.

In RCTs, the benefit is unknown to the patient because of the blinded nature of the study, which could make patients less adherent since there is no advantage for them as participants if they are in the placebo arm. Adherence may be improved in the outpatient setting where the patient knows that they will receive the benefits of taking PrEP as prescribed. This is why studying individuals in the outpatient setting is important to help assuage providers of the perceived risks.
**Risk Compensation**

The concerns about risk compensation with initiation of PrEP are based on the idea that behaviors increasing risk of HIV infection will become more prevalent because the patient perceives decreased susceptibility to HIV.\(^75\) This can be measured in several way including changes in numbers of partners, changes in condom usage, and STI acquisition. Through a survey of 164 MSM from New York City in committed relationships, Gamarel et al. found that individuals, who believe condoms interfere with intimacy, are more likely to initiate PrEP \((p<0.001)\),\(^76\) implying that people may use PrEP to avoid the “pleasure penalty” associated with condoms. If patients forgo the use of condoms, this act of risk compensation could undermine the protection conferred by PrEP and negate its usefulness as a preventative intervention.

Sexual behaviors that increase risk for HIV infections are a concern that providers cite in the Karris et al. study as a reason against prescribing it to at-risk populations\(^2\) and some of the media have gone so far as to stigmatize users as “Truvada whores.”\(^77,78\) The stigmatization may reduce the motivation of individuals to seek and/or continue PrEP, and the negative association may alter an individual’s perception of their own need or eligibility for PrEP. Insurance companies may even remove or decrease coverage of PrEP if risk compensation becomes synonymous with PrEP because it would make taking Truvada a less cost effective intervention, and less profitable for the insurance companies. Without coverage, the out-of-pocket cost for PrEP is estimated at $17,000/year.\(^77\)
Evidence of risk compensation was not observed in the iPrEx, iPrEx OLE, Partners, or TDF2 trials, which included over 5,000 participants receiving PrEP.\textsuperscript{3,4,5,6,7} In the iPrEx study, the number of self-reported partners decreased (from 11-12 down to 4-6), sexual encounters with condom use increased (50% of partners to 75-80% of partners), and STIs decreased in both the control and treatment groups.\textsuperscript{3} This study did not include statistical analysis for these indicators of risk compensation. This was also true in the iPrEx OLE cohort study. Grant et al. saw that self-reported number of sexual partners, incidence of URAIs (34\% to 25\%, p=0.006), incidence of unprotected insertive anal intercourse (UIAI), and STIs decreased during follow-up in both the untreated and treated groups.\textsuperscript{7} Unfortunately this study also did not include statistical data for these measures, so it is uncertain if they changed in a significant way.

Volk et al., with support from Kaiser Permanente, designed the first prospective cohort study of 801 MSM patients from San Francisco, California to determine the incidence of PrEP risk compensation in the setting of a PrEP outpatient clinic instead of a randomized control trial. Studying individuals in a setting that emulates how the general MSM population interacts with the healthcare system can provide better insight into what every-day risk compensation behaviors look like independent of the close scrutiny that comes with a randomized trial. Patients who initiated PrEP were screened for HIV and other STIs (gonorrhea, chlamydia, syphilis) every 1 to 3 months after initiation of PrEP. The mean follow-up was 7.2 months. Patients that started PrEP were more likely to report multiple sex partners (84\% vs 69\% p<0.001), but were not more likely to report having an HIV-positive partner (30\% vs 25\% p=0.18) compared to the non-initiators.
A subset of the PrEP initiators (n=188) was surveyed at 6 and 12 months regarding changes in sexual behavior. There was a 76% response rate. The survey found that the majority of patients reported an unchanged number of sexual partners (76%) and unchanged condom usage (56%). However, 41% reported decreased condom usage, which could be evidence of risk compensation. STI rates increased from 30% of PrEP users at 6 months to 50% of PrEP at 12 months, but no new HIV infections occurred. This study did not include statistical comparisons of the 6-month versus 12-month data, so we are unable to get a clear picture of the changes in risk behavior. This study also did not have a control group, which makes it hard to attribute these changes in behavior solely to PrEP. The findings from this study indicate that risk compensation could be a true issue in the outpatient setting.

The PROUD study also documented some signs of risk compensation. This open-label RCT in the setting of 13 sexual health clinics in England followed 544 HIV-negative MSM, who had condomless sex within 90 days of enrollment. The aim of the study was determine the time required to enroll 500 participants with a secondary aim to study HIV infection, safety, adherence, and risk compensation. Individuals were randomized to start daily PrEP (TDF 245mg, FTC 200mg) immediately (n=275) or to delay initiation for a year (n=269). The delayed group was offered access to PEP in the interim. Drug delay allows for the study of homogenous patients, i.e. both groups are interested to start PrEP, as opposed to a cohort study comparing non-initiators, who may have low-risk characteristics for HIV infection, and initiators, who may have higher-risk characteristics for HIV infection. They had follow-up every 3 months which included
HIV testing, STI screening (gonorrhea, chlamydia, and syphilis), questionnaires, and a diary review. Monthly questionnaires and daily diaries were used to report sexual behavior and medication compliance. Because of high incidences of HIV, individuals in the delayed group were started on PrEP 2 years after accrual started. The HIV risk reduction in the immediate treatment group was 86% (90% CI, 64-96). Of the five individuals who became infected with HIV in the immediate treatment group, 3 were found to be infected at baseline. Two of these three were found to have the Met184 mutation. The two additional participants, who were found to be infected with HIV later in the study, did not present with antiretroviral resistant HIV virus. The most common drug-related symptoms were nausea, headache, and arthralgia.79

McCormack et al. wanted to also study individual-level adherence and longitudinal sexual behaviors, but they lacked enough participants to complete the questionnaires and diaries. They were only able to study comparisons of baseline to one-year questionnaires. There was a proportion of individuals in the immediate PrEP group that reported URAI with 10 or more partners (21% VS 12%, p=0.03), which may be indicative of increased risk compensation, but it did not result in a significant difference between groups regarding number of sexual partners at 1 year (p=0.57) or number of STIs (p=0.74). Using rectal STIs as an indicator of condom usage was also not significantly changed (p=0.99).79

In the outpatient setting, the CDC recommends following up with individuals on PrEP every 3 months to check for HIV and other STI infections. With time constraints of short appointments and large patient panels, it is possible that providers may not have the
same amount of time or resources compared to clinical trials to educate and support each person initiating PrEP, which could lead to increased risk compensation or decreased compliance with PrEP. On the other hand, even though 3-month follow-ups in the clinic may not be as thorough as in a RCT, they may generate similar risk reduction by engaging at-risk population into the healthcare system.

More research on PrEP in the outpatient setting beyond the Kaiser Permanente and PROUD studies are needed to determine if risk compensation occurs in the general MSM population. Both of these studies lacked in-depth analyses of risk compensation behaviors. They also did not include any longitudinal data on adherence or side effects, which will be important for people who taking PrEP for decades. When these data become available, providers will have more information to further understand the risks and benefits of PrEP.
CHAPTER 3 – METHODS

Study Design

This will be an open-label randomized trial of PrEP in the outpatient setting.

Study Population and Sampling

The study population will draw from MSM within the Boston community who receive primary care at Boston Medical Center (BMC). Providers will use the CDC guidelines for PrEP to identify individuals who qualify as chemoprophylaxis candidates. Through a referral system, patients will be connected with PrEP clinic services. Patients will then be screened using selection criteria:

1. >18 years old
2. MSM
3. Currently sexually active
4. HIV-seronegative without clinical signs of acute HIV infection
5. Hepatitis B virus (HBV) and Hepatitis C virus (HCV) negative
6. Estimated CrCl >60 ml/min

Patients who are eligible will be randomized 1.) to start PrEP immediately as the CDC recommends or 2.) to delay initiation of PrEP by one year. The estimated sample size will be 526 individuals and will allow for the detection of a 10% decrease in condom usage between study arms. The study will have a power of 0.9 and a confidence level of 0.95.
**Treatment**

The immediate PrEP group will receive Truvada (200mg FTC, 300mg TDF), STI screening for chlamydia, gonorrhea, and syphilis, and education regarding medication compliance and condom usage. PrEP will be prescribed to be taken once daily and patients will receive PrEP free of charge. The delayed PrEP group will receive STI screening for chlamydia, gonorrhea, and syphilis and safe sex education for 1 year before initiating PrEP. Patients will be randomized to the immediate PrEP group or the delayed PrEP group using a web-based randomization. Subjects and investigators will not be masked to the results of the randomization.

**Study Variables and Measures**

This study will look at several variables related to risk compensation. We will track condom usage, HIV status, STI status (screening for penile, rectal, pharyngeal infections), and number of sexual partners. We will also be checking Truvada levels to monitor adherence to the medication. HIV and STIs status will be checked in-office. Partner number, number of sexual encounters, and condom usage will be followed in an online survey. Patients will be encouraged to keep a diary to help keep track of sexual behaviors.

**Recruitment**

Patients will be recruited through their primary care providers at BMC. If this does not yield enough participants, we will obtain permission to post flyers for the study around the BMC campus and South End community.
Study Protocol and Data Collection

This study will have participants attend 13 visits to the PrEP clinic. During the initial visit, patients will be screened for eligibility (see Study Population and Sample Size). Patients will be screened for HIV using the Architect HIV Ag/AB Combo test. Patients will also be screened for chlamydia and gonorrhea within the urethra, the rectum, and the pharynx using polymerase chain reaction (PCR). Serology testing will be completed for syphilis. HBV and HCV will be tested using serology and PCR. CrCl will be tested by obtaining a Chemistry 7 panel. Patients randomized to the immediate PrEP group will not be administered PrEP until they pass the eligibility screening.

Follow-up appointments will be focused on continued safe sex education, STI status, HIV status, and reporting of sexual partners, condom usage, and sexual encounters. Participants who become HIV-positive will be eliminated from the study and connected with the BMC HIV clinic to initiate cART if interested.

Initial visit

Immediate PrEP Group

1. Education – Discuss PrEP adherence and continued condom usage with HIV-positive partners
2. HIV testing
3. STI testing – syphilis, gonorrhea, chlamydia
4. HBV and HCV testing
5. Estimated CrCl testing
6. Retrospective questionnaire from the previous 3 months regarding:
   A. Condom usage
   B. Number of partners

7. Baseline serum will also be checked for evidence of cART

8. Administration of a 90-day supply of Truvada to be taken once daily after completing eligibility screening

**Delayed PrEP Group**

1. Education – Discuss continued condom usage with HIV-positive partners
2. HIV testing
3. STI testing – syphilis, gonorrhea, chlamydia
4. HBV and HCV testing
5. Estimated CrCl testing
6. Retrospective questionnaire from the previous 3 months regarding:
   A. Condom usage
   B. Number of partners

**Follow-up visit** – 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 33, 36 months

**Immediate PrEP Group**

1. Education – Re-education about PrEP adherence and continued condom usage with HIV-positive partners
2. HIV testing
3. STI testing/treatment
4. Retrospective questionnaire from the previous 3 months regarding:
   A. Number of partners
   B. Number and types of sexual encounters
   C. Number of times condoms were used

5. Serum sampling for evidence of therapeutic levels of Truvada

6. Administration of a 90-day supply of Truvada to be taken once daily

**Delayed PrEP Group**

1. Education – Patients will be re-educated about continued condom use

2. HIV testing

3. STI testing/treatment

4. Retrospective questionnaire from the previous 3 months regarding:
   A. Number of partners
   B. Number and types of sexual encounters
   C. Number of times condoms were used

*At 12 months, switch to the Immediate PrEP Group follow-up protocol

**Analysis**

263 participants will be in the immediate PrEP group and 263 participants will be in the delayed PrEP group. This sample size of 526 will allow for the detection of a 10% decrease in condom usage between study arms. The study will have a power of 0.9 and a confidence level of 0.95 (p=0.05).

For statistical analysis between the immediate PrEP group and the delayed PrEP group, analysis of variance (ANOVA) will be performed for survey responses regarding
number of sexual partners, number/types of sexual encounters, and condom usage. This statistical analysis will also be performed for contracted STIs confirmed by blood or sample lab tests. ANOVA will also be used to compare medication adherence between groups. Year one of the immediate PrEP group will be compared with year two of the delayed group.

ANOVA will be performed for the delayed PrEP group to analyze data from before (year 1 of study) and after the initiation of PrEP (year 2 and 3 of study) for number of sexual partners, number/types of sexual encounters, condom usage, and contracted STIs. This statistical test will also be performed for the immediate PrEP group to look at data from before (3 months previous to study) and after the initiation of PrEP (year 1, 2, and 3 of study) for all topics listed above with the exception of number/types of sexual encounters. Due to the retrospective nature of the survey, we would not expect someone to remember details about number/types of sexual encounters 3 month previous to the start of the study.

A Kaplan-Meier analysis will be used to compute the cumulative incidences of STIs and HIV at 0, 6, 12, 18, 24, 30, and 36 months.

**Timeline and Resources**

The study will most likely start in 2017 after identifying a location and staff for the PrEP clinic on the BMC campus. The clinic will employ 1 doctor (principle investigator (PI)), 2 physician assistants, 2 nurses, and a receptionist. This clinic will not require its own pharmacy or laboratory because of already existing infrastructure on-campus. This study will be overseen by the PI with assistance from his/her study
coordinator, who will coordinate patients in addition to completing data collection and data entry. This study will also require a statistician for data analysis.

Initial PrEP and safe sex education will completed by the physician assistant and subsequent education will be initiated by a nurse. STIs requiring swab samples for PCR will be obtained by the doctor or a physician assistant. STIs, HBV/HCV, and estimated CrCl clearance requiring blood samples will be obtained by a nurse.

It will likely take 3-4 years to accrue the 526 participants needed for the study. The study itself should take 3 years to finish plus an additional 6 months for data analysis and publication submission. Maximum time to completion will be <8 years.

**Institutional Review Board**

We intend to submit our proposal to the IRB at BMC. Our study will require a full-board review as it poses greater than minimal risk and does not meet the requirements for exemption or expedited review.
CHAPTER 4 – CONCLUSION

Discussion

This proposed research has several strengths. First, having an open-label rather than a placebo-controlled study allows for relevant data to be collected about the general MSM population on issues important to the success of an HIV prevention clinic. These things include the efficacy of PrEP and changes in sexual behavior of individuals on PrEP. Secondly, placebo-controlled studies may underestimate real-world adherence due to the fact that there is less incentive to take a pill that may be a placebo. This study will give a more realistic perspective on medication compliance. Thirdly, having participants in the study for 3 years will increase the longitudinal data on adherence and risk compensation with a rich data set. Lastly, this study has been designed to have low type 1 and type 2 errors. This means it is unlikely that a null hypothesis will be rejected when it is actually true and is unlikely that the study will fail to reject a null hypothesis when the alternative hypothesis is true.

One weakness of this study is the close observation of the participants. Adding too many interventions beyond regular outpatient visits to the PrEP clinic may influence the behaviors of the individuals in the study. Participants may want to improve the dynamics with their PrEP provider and be dishonest with their surveys regarding risk compensation. The hope is that this will not happen.

Some obstacles are anticipated. It may be difficult to procure the funding required to start a PrEP clinic. Some funding will come from research grants to support the study, but additional financial support will be needed from BMC to make this a reality. This
may delay when the project can begin. It is also uncertain if the demand exists to have a stand-alone PrEP clinic, which may result in an insufficient number of participants for the research study. PrEP administration may be better handled by primary care providers in an outpatient clinic setting.

The Boston area provides an immense amount of diversity, so this study will be generalizable to MSM populations throughout the United States. Because this study only looks at the MSM population, it is unlikely to be generalizable to heterosexual individuals or IVDUs.

Summary

HIV affects over 1.2 million people in the United States. This viral infection leads to decreased CD4+ T cells, increased susceptibility to OIs, and eventually AIDS and death.\(^{80}\) Viral loads can be decreased to undetectable levels with the use of cART. Every year 50,000 individuals become newly infected with HIV in the U.S.,\(^{23}\) this may be reduced in the future due to the FDA approval of Truvada for the indication of PrEP. When detectable levels of drug are present in the blood stream of patients, there is up to a 92% relative risk reduction of HIV infection compared to placebo.\(^{3}\)

Because PrEP treatment has only been approved since 2012, there are many perceived barriers to administration of PrEP to patients. Side effects, PrEP adherence, development of resistant virus, and risk compensation are several of the ongoing concerns that providers cite as reasons against prescribing PrEP to at-risk populations.\(^{2}\)

Most publications on PrEP have been randomized placebo trials in the research setting that focused on adherence-based efficacy\(^{3}\) and risk compensation behaviors.\(^{3,4}\)
Now that Truvada is widely available, researchers are starting to elucidate patients’ sexual habits on PrEP in the everyday outpatient settings. However, there is a need for more research regarding how the general population will utilize PrEP, specifically to determine if risk compensation occurs and what it looks like over extended periods of time. There is also a need for the implementation of a PrEP clinic as there are none in the Boston area despite the large number of at-risk individuals in Massachusetts.

This study initiates a PrEP clinic at BMC to follow patients for 3 years in an open-label randomized controlled trial of eligible MSM patients who either start PrEP immediately or are delayed by one year. The aim is to determine if risk compensation occurs in these patients by following condom usage, development of STIs, sexual partner numbers, and sexual encounter numbers/types. Secondary outcomes will include measurements of medication adherence. The resulting information will help the medical field better understand the longitudinal risks and benefits of PrEP and what they can do to improve compliance and risk-reduction behaviors.

**Clinical and/or Public Health Significance**

PrEP is covered by Medicaid and often covered by private insurers, so the remaining barriers to accessing PrEP are three-fold. Patients may be unaware of PrEP or their eligibility, there may be nowhere to obtain it, or providers are uncomfortable prescribing it. Every doctor before entering the healthcare system takes the Hippocratic Oath, which includes commitment to do no harm. From the surveys it seems that providers hesitate to prescribe PrEP because there is not enough data about the risks and
benefits to be able to make an informed decision for their patients, who rely on them to do so.

This study addresses at least two of these barriers. Initiating a clinic gives at-risk people a place to go to receive PrEP. It also supplies the providers with a wealth of information about adherence and risk compensation over an extended period of time to help them with medical decision making. Indirectly, starting a PrEP clinic may increase the awareness of PrEP as individuals start to visit and information spreads by word of mouth.

HIV is a significant public health issue around the world which kills over 1 million people every year. The ability to prevent this viral infection from occurring can prevent millions of premature deaths. PrEP offers one significant platform to achieve this public health intervention, and may serve as a great tool in the fight against HIV.
## LIST OF JOURNAL ABBREVIATIONS

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<td>AIS</td>
<td>Association of Interdisciplinary Studies</td>
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<td>AM J Public Health</td>
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<td>Lancet Infect Dis</td>
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<td>International AIDS Society</td>
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REFERENCES


http://cid.oxfordjournals.org/content/58/5/704.abstract.


43. Wild CT, Shugars DC, Greenwell TK, McDanal CB, Matthews TJ. Peptides corresponding to a predictive alpha-helical domain of human immunodeficiency virus type 1 gp41 are potent inhibitors of virus infection. Proc Natl Acad Sci U S A. 1994;91(21):9770-9774.


71. CDC stacks | laboratory testing for the diagnosis of HIV infection : Updated recommendations - 23447 | guidelines and recommendations  


79. McCormack S, Dunn DT, Desai M, et al. Pre-exposure prophylaxis to prevent the acquisition of HIV-1 infection (PROUD): Effectiveness results from the pilot phase of a

CURRICULUM VITAE

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EDUCATION

- Boston University School of Medicine, Boston MA
  Master of Science, anticipated 2016
  Physician Assistant
  2014 - currently

- Johns Hopkins School of Public Health, Baltimore MD
  Master of Science, September 2010
  Biochemistry and Molecular Biology, GPA: 3.74
  2008 - 2010

- Wittenberg University, Springfield OH
  Bachelor of Arts, May 2007
  Dual Major: Biology and Religion, GPA: 3.55 Cum Laude
  2003 - 2007

- National University of Ireland, Galway Ireland
  Study Abroad
  Spring 2006

CERTIFICATIONS

- ACLS, American Heart Association
  2015

CLINICAL AND LAB RESEARCH EXPERIENCE

- Johns Hopkins School of Medicine, Research Program Coordinator, Baltimore, MD
  -Principle Investigator: Dr. Robert Siliciano, Division of Infectious Disease
  -Study of HIV infection and the latent viral reservoir
  2013 - 2014

- Johns Hopkins School of Medicine, Research Technologist, Baltimore, MD
  -Principle Investigator: Dr. Naresh Punjabi, Division of Pulmonary and Critical Care Medicine
  -Study of sleep apnea and its molecular implications on type two diabetes
  2012 - 2013

- Johns Hopkins School of Public Health, Graduate Researcher, Baltimore, MD
  -Principle Investigator: Dr. Jürgen Bosch, Department of Biochemistry and Molecular Biology
  -Study of Plasmodium invasion machinery using protein crystallography techniques
  2009 - 2010

- Wittenberg University, Student Researcher, Springfield, OH
  -Principle Investigator: Dr. Kevin Gribbins, Department of Biology
  -Histology of testis in Scincella lateralis
  2006 - 2007

PUBLICATIONS


PUBLICATIONS (con’t)

PRESENTATIONS
- **Boston University School of Medicine** 2016
  -Physician Assistant Student Grand Rounds
  Title: Ow, My Back
- **Boston University School of Medicine** 2015
  -Physician Assistant Student Grand Rounds
  Title: MitraClip
- **Johns Hopkins School of Public Health**, Baltimore, MD 2010
  -Biochemistry and Molecular Biology Conference
  Title: To Invade or Not to Invade

TEACHING EXPERIENCE
- **Johns Hopkins School of Public Health**, Teaching AssistantBiochemistry – An Introductory Course I and II, Professor: Randy Bryant 2009

ACADEMIC ACHIEVEMENTS
- Wittenberg University Honors Program 2004 - 2007
- Beta Beta Beta, national biological research society 2006 - 2007
- Omicron Delta Kappa, national society for scholarship/leadership 2006 - 2007
- Chi Alpha Sigma, national society for varsity athletics/scholarship/character 2006 - 2007
- NFHCA National Academic Squad, for student-athletes with a +3.0 GPA 2003 - 2006

ACADEMIC ORGANIZATIONS
- American Academy of Physician Assistants (AAPA) 2014 - currently
- Carl Tony Student PA Society
  Graduate Medical School Student Organization Representative 2014 - currently
  Educational Policy and Curriculum Committee 2014 - currently
  Graduation Committee 2016

ATHLETIC ORGANIZATIONS
- Baltimore Field Hockey Association 2008 - 2014
- North Jersey Field Hockey Association 2007 - 2008
  -North Coast Athletic Conference (NCAC) Tournament 2003 - 2006
  -NCAA Division III Tournament 2003 and 2004

COMMUNITY SERVICE
- Johns Hopkins Bayview Hospital, Patient Representative, Baltimore, MD 2011
  -Assisted patients with comfort needs
- Community Hospital Surgery Waiting Room, Springfield, OH 2007
  -Informed patients’ families about surgery progress

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