View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Yale University

Yale University
EliScholar – A Digital Platform for Scholarly Publishing at Yale

Yale Medicine Thesis Digital Library

School of Medicine

11-15-2006

Patterns of Resistance to Antiretroviral Therapy among HIV+ Patients in Clinical Care

Akash Shah

Follow this and additional works at: http://elischolar.library.yale.edu/ymtdl

Recommended Citation

Shah, Akash, "Patterns of Resistance to Antiretroviral Therapy among HIV+ Patients in Clinical Care" (2006). *Yale Medicine Thesis Digital Library*. 291.
http://elischolar.library.yale.edu/ymtdl/291

This Open Access Thesis is brought to you for free and open access by the School of Medicine at EliScholar – A Digital Platform for Scholarly Publishing at Yale. It has been accepted for inclusion in Yale Medicine Thesis Digital Library by an authorized administrator of EliScholar – A Digital Platform for Scholarly Publishing at Yale. For more information, please contact elischolar@yale.edu.

Patterns of Resistance to Antiretroviral Therapy among HIV+ Patients in Clinical Care

A Thesis Submitted to the
Yale University School of Medicine
in Partial Fulfillment of the Requirements for the
Degree of Doctor of Medicine

by

Akash D Shah
2006

PATTERNS OF RESISTANCE TO ANTIRETROVIRAL THERAPY AMONG HIV+ PATIENTS IN CLINICAL CARE. Akash D. Shah and Michael J. Kozal. Section of Infectious Diseases, Yale AIDS Program, Yale University School of Medicine, New Haven, CT.

HIV-1 antiretroviral resistance has posed major challenges to treatment advances of the last decade. However, few studies have analyzed the prevalence and time trends of drug resistance among HIV+ patients on antiretroviral therapy followed longitudinally in clinical care. The purpose of this study is to determine the cumulative prevalence of HIV genotypic drug resistance and the dynamics of resistance development in HIV+ patients in care. We hypothesized that a >5% increase in resistance would occur per 6-month period and a >15% increase in drug resistance would occur over 18 months.

This retrospective longitudinal study consisted of patients from the two largest HIV clinics in Connecticut who were enrolled in the Options Project Study from 2000-2003. HIV+ patients were consented and enrolled in the resistance substudy. HIV genotypic resistance testing was done on plasma samples available for each patient at study baseline and at ~6 month intervals for 18 months. HIV viral load and resistance data were matched to behavioral and demographic data for each patient. Genotypic drug resistance was defined according to the International AIDS Society 2004 guidelines. The chi-square test for linear trends was used to assess resistance trends.

396 HIV+ patients enrolled in the study and had archived plasma available for analysis. The cumulative prevalence of drug resistance increased from 32.1% to 46.3% for patients with 18 consecutive months of data, 31.9% to 50.7% for patients with 12 consecutive months of data, and 30.2% to 41.3% for patients with 6 consecutive months of data.

During the period of study for this HIV+ patient care population, the cumulative prevalence of HIV genotypic drug resistance rose dramatically. The findings emphasize the need for addressing antiretroviral resistance in a clinical setting through physician education, reduction of transmission risks, regimen adjustments, newer agents, and utilization of genotype testing.

ACKNOWLEDGMENTS

I would like to extend my most sincere thanks to my mentors and collaborators on this project. I am deeply grateful to my sponsor, Michael Kozal, MD, for his teaching, support, time, and encouragement throughout the course of this project. I thank Jennifer Chiarella and Rivet Amico for helping with the organization and compilation of data for this project. I thank Tassos Kyriakides for his support and advice on statistical analyses. Lastly, I would like to thank the Office of Student Research at Yale University for financial support toward the completion of this research.

TABLE OF CONTENTS

Introduction…….1

Statement of Purpose…….12

Methods……..14

Results…...…….22

Discussion……..38

References……..45

INTRODUCTION

As one of the most devastating epidemics in recent history, Acquired Immunodeficiency Syndrome (AIDS) has killed approximately 25 million people since its recognition in 1981. An estimated 40.3 million persons were living with HIV/AIDS in 2005. There were 4.9 million new HIV infections and 3.1 million deaths from AIDS in 2005 (1). In the United States, an estimated 1.1 million persons were living with HIV/AIDS at the end of 2003. Approximately 40,000 new HIV infections occur every year in the United States (2). The development of antiretroviral therapy has led to a major reduction in mortality due to HIV disease. However, enthusiasm for these therapeutic advances of the past decade has been tempered in recent years by the development of resistance to antiretroviral therapy.

Advent of Antiretroviral Therapy

In the years immediately following the discovery of HIV, opportunistic infections were largely responsible for the high mortality rate among AIDS patients. With the lack of therapeutic options, 85% of AIDS patients died within five years (3). In 1987, zidovudine became the first drug introduced for treatment of HIV. Zidovudine is a nucleoside analogue that inhibits HIV-1 reverse transcriptase, one of the proteins essential for viral functionality and induces chain termination of proviral DNA through competitive inhibition. More nucleoside reverse transcriptase inhibitors (NRTIs) were introduced four years later; however, deaths from AIDS continued to increase as scientists recognized the quick development of resistance among patients on monotherapy with NRTIs (4, 5, 6). An improved understanding of the virus itself led to

the discovery of protease inhibitors (PIs) in 1995. Mature viral proteins are cleaved from longer polypeptides by the HIV protease enzyme, a necessary step in the viral life cycle that results in the production of infectious virions. Protease inhibitors prevented the formation of these mature virions. In 1997, scientists recognized the effectiveness of triple therapy, otherwise known as highly active antiretroviral therapy (HAART). HAART is generally considered an antiretroviral regimen that contains 3 drugs with at least one drug from two different antiretroviral classes. Mortality of patients with AIDS was greatly reduced. HAART was effective in suppressing HIV-1 viral load and reducing viral replication, thus minimizing the chances of developing resistance to therapy. Non-nucleoside reverse transcriptase inhibitors (NNRTIs) were also introduced. NNRTIs differed from NRTIs in their mechanism of action. NNRTIs bound to a site distant from the active site for reverse transcriptase, leading to conformation changes in the active site and inhibition of DNA synthesis (7).

Antiretroviral Resistance

The development of antiretroviral therapy for HIV ushered in a new era of treatment and management of the disease. While antiretrovirals have lengthened survival and slowed disease progression, poor adherence due to complexities and toxicities of treatment regimens, insufficient regimen potency, and the biology of HIV-1 itself have contributed to the development of resistance. The persistence of drug-resistant viral variants despite therapy has played a significant role in the direction of the HIV epidemic. Resistance to zidovudine was first described in 1989 by Larder B *et al.* (4) with mutations in the HIV-1 reverse transcriptase gene (8). Of individuals on ddI

monotherapy, 60% developed resistance within 6 months of treatment (6). An estimated 30-50% of individuals on HAART were shown to have developed drug-resistant mutations within 2 years of therapy (9).

Viral drug resistance is caused by the selection of viral variants with mutations during viral replication that persists despite antiretroviral therapy. The high viral replication rate of HIV-1 (an estimated 10^9 virions are produced daily (10)), the poor fidelity of reverse transcriptase for RNA polymerase, and the lack of a viral enzyme proofreading capability lead to a high mutation rate in the *pol* gene (the gene that encodes the reverse transcriptase and protease enzymes), estimated at ~ one mutation per replication. These viral dynamics ensure that viral replication that occurs during therapy will result in the development of drug resistance and eventual virological rebound. Although some viral mutations may have a negative impact on the replication capability of the virus (11), HIV has demonstrated the ability to function despite multiple resistance mutations. Further, the virus will continue to develop mutations in the face of drug pressure. These additional mutations, termed compensatory mutations, help increase the virus's replication capability in the face of antiretroviral therapy (11).

Viral Latency

Although the dominant viral population in an individual at any given time may show no drug resistance, the high replication and mutation rates of HIV-1 point to the likelihood that subpopulations of the virus exist that contain perhaps all possible mutations (12). It has been shown that viral populations in the CNS and semen exist independently of populations found in blood cells and these body compartments can

harbor viral variants with resistance (7, 13). An estimated 10^7-10^8 infected cells can exist in lymphoid tissues in a steady state (14). Given the short half-life of infected cells, the virus must have a high replication rate, with resulting mutations contributing to a diversity of viral quasispecies. Because HAART often does not inhibit viral replication completely, latent infections in resting memory CD4+ T cells, macrophages, and monocytes, which can survive for long periods of time, contain many quasispecies that can reemerge once the suppressive pressure of HAART is removed (10).

Risk Factors for Antiretroviral Resistance

Drug resistance in HIV can emerge 1) with a failure to suppress HIV-1 RNA to nondetectable levels 2) with subtherapeutic plasma and intracellular drug levels due to a lack of adherence from regimen complexities/toxicities, borderline pharmacokinetics, and drug-drug or drug-food interactions and 3) with non-potent regimens e.g. sequential monotherapy; dual therapy or single class therapy (12). Resistant strains do not necessarily lead to virological failure (unless a patient is primarily infected with resistant strains – this leads to failure if not recognized), but instead develop in the setting of treatment failure characterized by quantities of drug both effective enough to exert a positive selective pressure but not sufficient enough to inhibit viral replication completely (15). In a population-based study of individuals followed for 30 months after initiation of HAART therapy, high baseline plasma viral loads, history of injection drug use, and <95% adherence were associated with drug-resistant mutations (15). The association with high baseline plasma viral loads likely reflects incomplete viral suppression as well as the

greater presence of minority HIV-1 quasispecies, and a larger pool of latently infected cells (and acutely infected cells) in these individuals.

Of note, the association between drug resistance and adherence is thought to follow a bell-shaped curve. The presence of antiretroviral drugs creates a selective pressure for drug-resistant mutations to develop. At the same time, high levels of adherence can suppress viral replication and inhibit development of drug resistance. In a study of 87 patients on protease inhibitor therapy, the peak rate of drug-resistant mutations was 2.08 over 12 months in patients that had 100% adherence but still had viremia – active viral replication in the face of non-fully suppressive drug exposure. However, 48% of these patients had nondetectable VL (VL<50), reducing this rate to 1.08 mutations. When considering both viremic and nonviremic patients, the peak rate of drug-resistant mutations occurred at 87% adherence (16). This suggests that a high level of adherence is associated with decreased risk of resistance due to the percentage of highly-adherent patients with nonviremia. When the assumed viral suppression in 100%-adherent patients was 95%, the peak rate of protease-inhibitor resistance was calculated to occur at 45% adherence. Thus, to clarify the association between drug resistance and adherence, drug resistance is more common in patients with simultaneously high adherence levels and incomplete viral suppression. Other studies have determined that this bell-shaped curve may not be accurate for NNRTI-resistance. Recent data from Bangsberg and colleagues suggest the drug resistance to NNRTIs can occur with as little as 2% adherence (17).

Prevalence of Antiretroviral Resistance

In the era of HAART therapy, the development of drug resistance has been aided by low adherence, subtherapeutic drug levels, and transmission of resistant viral strains as patients live longer and healthier lives and continue to engage in HIV transmission risk behaviors which include unprotected sex and the sharing of injection drug needles/works. Resistance occurs not only in chronically infected patients on treatment, but also in newly infected people without treatment due to the exposure to drug resistant strains during the transmission event.

In a study of newly-infected patients between 1989 and 1998 with a history of HIV seroconversion in the preceding twelve months and less than seven days of total antiretroviral therapy, resistance was measured by phenotypic drug susceptibility reduction. Two percent of patients had a greater than 10-fold reduction in susceptibility to one or more drugs and resistance was confirmed by genotypic sequence testing for drug-resistant mutations. Twenty-six percent of patients had a 2.5 to 10-fold reduction in susceptibility (18). A study of drug-naïve patients from 10 cities between 1997 and 2001 showed 8.3% of patients with RTI- or PI-resistant mutations. The prevalence of resistance differed by sexual orientation (11.6% among men who had sex with men vs. 4.7% among heterosexual men), race (13.0% among whites vs. 5.4% among African-Americans and 7.9% among Hispanics), and status of sexual partners (15.2% among patients whose partners were on antiretroviral therapy (19). Figure 1, adapted from Bennett D *et al.* (20) shows the most recent data from the CDC presented at CROI in 2005 on drug resistance in newly diagnosed treatment-naïve patients by demographics and drug class.

Prevalence of HIV Drug Resistance

CDC data on prevalence of drug resistance in 787 newly diagnosed treatment-naïve patients from 89 sites in 6 states (2003-2004)

Demographics

Men	14.9%
Women	13.8%
MSM	15.9%
Hetero	13.0%
IDUs	14.1%
Black	14.2%
Hispanics	13.6%
Whites	15.3%

Drug Class

Any class	14.5%
NRTI	7.1%
NNRTI	8.4%
PI	2.8%
>2 classes	3.1%

Figure 1. Prevalence of drug resistance in newly diagnosed ART-naïve subjects by demographic and drug class

In chronically HIV-infected, treatment-naïve patients, a 10.8% prevalence of resistance was found in one cohort. There was a 40% increase per year in prevalence of drug-resistant mutations in cohorts analyzed from 1999 to 2001 (21).

From a representative sample of 1800 HIV-infected patients receiving care in 1998, it was estimated that 76% of HIV-infected adults with viremia (VL>500 copies HIV RNA/ml plasma) at the time were resistant to at least one antiretroviral drug. Higher levels of resistance were independently associated with current HAART therapy, lowest reported CD4 count, and high viral load. Of note, the reported prevalence was among patients with detectable viral load. If all patients without a detectable viral load were assumed to harbor non-resistant virus, the prevalence was still 48%. This analysis provided an important description of the high prevalence of resistance among patients in the early years of HAART (22), when many patients were the survivors of serial monotherapy with drugs such as AZT and ddI, leading to resistance.

Historical Genotypes

Because of the possibility of archived mutations in latently infected T-cells, cross-sectional genotyping, which does not take into account the genotypic history of an individual, can underestimate the number of drug-resistant mutations present. A single genotypic analysis analyzes the dominant viral strain circulating in a patient's plasma. However, true burden of resistance would be underestimated if a patient harbored a subpopulation of drug-resistant virus that was actively being suppressed due to sufficient therapeutic suppression with long-term therapy. In a retrospective study of HIV patients in British Columbia who had undergone at least 3 genotyping tests between 1996 and 2004, a historical genotype profile was created for each patient and compared to the results of the most recent genotype test as well as the most recent genotype on therapy for each patient. Prevalence of drug-resistant mutations was higher when the genotypic history was taken into account than only in the most recent genotypic test. For example, 14% of subjects had the PI L90M mutation in their genotypic history vs. 8% in their most recent genotype. For NRTIs, 58% of subjects had the M184V mutation in their genotypic history vs. 24% of subjects with this mutation in their most recent genotypic test. For all drug-resistant mutations combined, a 12% historical prevalence of resistance was underestimated at 5% using only the most recent genotypic test. Thus, historical genotypic testing, which accounts for more possible drug-resistant mutations in an individual, is more likely to account for the prevalence of resistance in a population. Historical genotyping, which takes into account the presence of archived mutations, is important to note in any patient as subpopulations of drug-resistant virus can reemerge quickly with resumption of or changes to therapy (23).

Viral Fitness

Drug-resistant mutations can impair viral fitness by lowering the enzymatic efficiency of target enzymes resulting in an inefficient process of replication. CD4 counts have been shown to remain stable despite the development of resistance, and continue to decline once the selective pressure of therapy is removed (24). While this may suggest the possibility of residual drug function despite resistance, the lower virulence of the resistant virus may also contribute to a stable CD4 count (18).

Time Trends in Prevalence of Antiretroviral Resistance

Prevalence of HIV drug resistance in the newly infected population has been rising over time. In a study of newly-infected patients from ten North American cities, the prevalence of drug-resistance detected by 10-fold reductions in drug susceptibility 3.4% in 1995 to 12.4% in 2000, and as tested by sequence analysis increased from 8.0% in 1995 to 22.7% in 2000. The frequency of multi-drug resistance increased from 1.1% to 6.2% by phenotypic susceptibility testing and 3.8% to 10.2% by sequence analysis during the same period. While the results vary based on the method of testing for resistance, the results clearly suggest increasing transmission of drug-resistant virus over time (25).

Resistance to different classes of drugs has been shown to increase at different rates. A case series study of 225 patients with recent HIV-1 infection between 1996 and 2001 at a hospital in San Francisco found an increase of resistance to NNRTIs from 0% in 1996 to 13.2% in 2001. This likely reflects the increasing prevalence of NNRTIs

during the latter half of this period. Also, NNRTI-resistance can develop more easily from one-step high-level mutations which greatly reduce susceptibility. Resistance to PIs remained stable during this period (26).

Clinical Implications of Resistance

Development of antiretroviral resistance has been associated with decreased treatment options for patients (18, 27). It has also been associated with clinical progression of disease and mortality. In a study of 1388 ART-naïve patients in Canada who initiated HAART therapy from 1996-2000, all-cause mortality was followed. Emergence of ART resistance, a higher baseline VL, and decreased adherence were among the factors associated with an increased risk of death. Resistance to any drug represented a hazard ratio of 1.8. Resistance to non-3TC NRTIs represented a hazard ratio of 2.93 (28).

In a retrospective analysis of patients from the AIDS Clinical Trials Group protocol with zidovudine-resistance, associations between zidovudine resistance and HIV-1 isolate synctium-inducing phenotypes, CD4 count, HIV-1 disease stage, and treatment were analyzed. The synctium-inducing phenotype, signifying a multinucleated cell mass, predicts clinical and immunologic deterioration. High-level zidovudine resistance in this study predicted accelerated disease progression and death. Moderate resistance was not similarly associated with these outcomes. Importantly, other predictors of progression, including synctium-inducing phenotype, baseline CD4 count and diagnosis of AIDS were controlled for in this study. While synctium-inducing phenotype predicted mortality, it was not associated with high-level zidovudine resistance (29).

Studies have also found associations between antiretroviral resistance and CD4 count and viral burden (5, 6). In a retrospective study of patients switched from zidovudine and didanosine with subsequent development of a didanosine-resistant mutation, significant decreases in CD4 count and increase in HIV RNA levels were seen (6). In a trial measuring phenotypic susceptibility in NRTI-experienced patients, reduced phenotypic susceptibility at baseline predicted virological failure (30).

Resistance Testing

Due to the negative clinical impact of resistance development, testing for resistance in clinical settings has been recommended in patients experiencing virological failure on a stable antiretroviral regimen as well as treatment-naïve patients acutely infected with HIV. With the high prevalence of resistance in newly-infected HIV+ patients, susceptibility testing is to be strongly considered (19, 20). Testing is also likely useful in treatment-naïve patients chronically infected with HIV. In chronically-infected HIV+ patients, testing is generally considered helpful in populations with a high prevalence of resistance. Genotype testing is in fact considered cost effective in most clinical scenarios (acute, chronic, pregnancy, etc.) (12).

It is clear that HIV drug resistance is a major problem both in treatment-naïve and treatment-experienced HIV-1 infected populations. A better understanding of the dynamics of resistance development and the overall burden of resistance among patients in clinical care is needed to better design strategies to prevent the development of and treat drug-resistant strains.

STATEMENT OF PURPOSE

In this setting of increasing resistance and continued risk behaviors, development and transmission of drug-resistant virus have become important points of focus in current research on HIV. Recent clinical trials have documented the prevalence of resistance over time in newly and chronically infected patients. While these studies have documented a rising prevalence of antiretroviral resistance in large HIV patient populations, there have been no clinic-level studies on the change in prevalence of antiretroviral resistance in HIV+ patients. Little data exists on the prevalence and time trends of HIV resistance among HIV+ patients for whom antiretroviral agents are prescribed and who are being followed longitudinally in clinical care. Previous studies have shown that this population is a likely source of continuing transmission of drug-resistant virus (31, 32). A better understanding of the dynamics of resistance over time, the development of new mutations, and associations between resistance and patient characteristics in this population will aid in the practical approach to HIV+ patients in clinical care.

Hypothesis

Previous literature has documented both the transmission of drug-resistant virus among all patient groups and continued risk behaviors in clinic populations. Given the presence of archived mutations in latently infected T-cells, the hypothesis in this study is that the cumulative prevalence of ART resistance, based on historical genotype results, in the clinic population to any ART class (reverse transcriptase inhibitors (RTI), non-nucleoside RTI (NNRTI), protease inhibitors (PI)) will increase at a rate of >5% per 6-

month interval. The cumulative rate of increase during the 18-month period of data in this study will exceed 15%. The importance of this hypothesis is that an increasing and significant number of individuals within the cohort will develop the potential for transmission of resistant virus over time.

METHODS

This study is an extension of the Options Project. The Options Project is an NIMH-funded study from which biologic data has been collected towards the investigation of antiretroviral resistance, adherence, and transmission risk behavior. The central focus of the Options Project is to analyze the effect of a physician-delivered intervention aimed to reduce HIV transmission risk behaviors in the setting of clinical care of HIV patients (33). Data was collected at baseline and follow-up visits on transmission risk behavior, length of time on therapy, gender, age, educational level, income level, support resources, welfare use, sexual orientation, duration of HIV, race, CD4, viral load (VL), and HIV risk factor. The present study is part of a supplemental substudy of the Options Project specifically focused on viral resistance development.

Delineation of Work

This study represents an analysis of demographic and resistance data obtained from patients in the Options Project resistance substudy. Patient recruitment for the resistance substudy, sample collection, and laboratory resistance testing were completed by Dr. Michael Kozal's team. Data organization was completed by Akash Shah with the assistance of Michael Kozal, MD, Jennifer Chiarella, and Rivet Amico, PhD. Data analysis and results were completed by Akash Shah with the assistance of Michael Kozal, MD for data interpretation. Statistical analysis was completed by Akash Shah with the assistance of Michael Kozal, MD and Tassos Kyriakides, PhD.

Patient Population

A total of 492 HIV+ patients were enrolled in the Options Project from the Nathan Smith Clinic (NSC) at Yale New Haven Hospital (YNHH) in New Haven, CT (n=247) and the Community Care Clinic at Hartford Hospital (HH) (n=245). Subjects had to provide written informed consent, be at least 18 years old, be healthy enough to complete procedures of the study, and show no obvious signs of dementia. The study was approved by the Yale University Human Investigations Committee, University of Connecticut IRB, and Hartford Hospital IRB. 451 patients consented to participate in the voluntary viral resistance substudy involving genotypic analysis between 2001 and 2003. Of these patients, data was obtained for 396 patients at YNHH (n=192) and HH (n=204).

The Nathan Smith Clinic at YNHH, serving patients in a 60-mile radius surrounding New Haven, CT, provides clinical care to approximately 1000 HIV+ patients. During the data collection period of the present study, 15 attending physicians, fellows, and physician assistants from the Infectious Diseases section at Yale School of Medicine provided care at the clinic. Within the clinic population, 35% of patients at the clinic are women. 45% of the patients are Caucasian, 40% are African-American, and 15% are Hispanic. A history of intravenous drug use (IDU) is the most common risk factor for acquisition of HIV among these patients at 45% of patients, followed by 30% with male same sex contact (MSM) and 25% with heterosexual contact.

The Community Care Clinic at HH also provides care to approximately 1000 HIV+ patients in the Hartford, CT region. 5 physicians and 2 nurse practitioners from the Infectious Diseases section at HH provide clinical care. Patient demographics are similar at NSC and the clinic at HH. 35% of patients at the clinic are women. The clinic has a

greater population of Hispanics at 35% than NSC. An additional 35% of patients are African-American and 30% are Caucasian. Risk factors for HIV acquisition are similar to NSC, with 44% reporting a history of IDU, 24% reporting MSM, 22% reporting heterosexual contact, and 10% unknown.

Plasma Sample Collection

All patients enrolled in the Options Project had plasma samples routinely drawn for measurement of VL and CD4 approximately every 3 months as part of the standard of clinical care. Approval was obtained to utilize the remaining specimens for resistance testing. Thus, no additional plasma specimens were required with enrollment in the resistance substudy. Unused specimens were saved for one year, and approximately 1-4 ml of plasma was still available after VL testing for each sample. Specimens from NSC were transported to the testing laboratory weekly. Specimens from HH were shipped when a sufficient sample size had accumulated in order to save costs.

Genotype data was recorded from plasma samples available for each patient at the first visit at the initiation of the study (baseline) and at approximate 6-month intervals for 18 months. HIV genotyping was not performed in samples with a nondetectable (ND) HIV VL as current genotyping assays require a detectable VL. ND samples are recorded as such. Samples with VL<1000 HIV RNA copies/ml are suboptimal for genotype testing. However, an ultracentrifuge step added to the assaying method allowed for a genotype result on approximately 75% of samples with VL between 50 and 1000 HIV RNA/ml. As the resistance substudy was supplemental, samples were not available for

every patient at every time point as genotyping was not required in the initial Options Project study.

Of note, enrollment into the study was staggered, and each patient's baseline plasma sample may have been drawn on different dates during the study period. Because this study is an analysis of drug resistance in continuing clinical care relative to a single point in patients' clinical care, the specific baseline dates do not affect the data.

Resistance Testing

Standard methods for DNA sequencing were applied in this study to detect genotype resistance in the virus. Standard ABI sequencing consists of *pol* gene isolation and sequence determination (34). Cryopreserved (-70° C) plasma aliquot samples were ultracentrifuged for one hour at -4° C. HIV-1 RNA was extracted from these samples using standard RNA isolation procedures. RT-PCR was used to isolate HIV-1 nucleic acid and PCR using HIV PRT primers, HIV PRT Plus reaction assay conditions, and 6 primer sets was utilized for standard ABI sequencing. See Kozal *et al*. (34) for specific details on this sequencing methodology.

Data Analysis

The primary aim of the study is to assess the change in prevalence of any genotypic resistance over time. The study is powered to detect this outcome. Based on the original sample size of 451 patients, the following assumptions are made in estimating the power: 1) baseline viral resistance of 24% 2) a projected increase in viral resistance of 10% over the 18-month period 3) drop-out rate of 5% over the 18-month

period. With a Type I error of 0.05, the power to detect a 10% increase at 18 months as compared to baseline is 79%. The power to detect a 15% increase is 99%.

Initially, the proportion of patients with 1-class, 2-class, and 3-class resistance, ND VL, and wild-type genotype (WT) was calculated at each time point. To arrive at a complete picture of how resistance changes in this clinic population based on historical genotype, various methods were utilized. Due to incomplete follow-up with all patients, the number of patients with plasma samples available at baseline and at each 6-month follow-up time point differed. For each patient with resistance data, an identification of all time points with available resistance data and number of consecutive time points with available resistance data were recorded. In order to analyze changes in prevalence of resistance over time, only those patients with at least two consecutive time points of genotype data available were analyzed. Patients were divided into three groups: 1) patients with 4 consecutive time points with complete genotype data (18 consecutive months) 2) patients with at least 3 consecutive time points with complete genotype data (any 12 consecutive months) and 3) patients with at least 2 consecutive time points with complete genotype data beginning at baseline (baseline and 6 months). In group 2, patients' baselines were redefined as the first time point for which the patient had genotype data available.

Within each group of patients, the following was derived and calculated from the data: 1) proportion of patients with ND VL, WT genotype, back-revertants (defined as patients with polymorphisms at RT codon 215), 1-class resistance, 2-class resistance, 3-class resistance, and total resistance (sum of patients with resistance to at least one drug class or revertant status). For purposes of this study, patients with ND VL were assumed

to have not developed resistance at that time point. The incidence of resistance was noted, and cumulative total resistance was calculated for each time point after baseline. The accumulation of additional new mutations was not analyzed once the first evidence of a drug-resistant or revertant mutation was documented. The analysis was also carried out excluding revertants.

Measurement of Resistance

Resistance was defined as the presence of at least 1 major drug resistance mutation based on guidelines from the International AIDS Society 2004 (35). NRTI back-revertants from earlier mutations at sites such as 215 C/D/E/S and 69 A/N/S in the *pol* gene were included as resistant as they likely represent a past presence of complete resistance to NRTIs with removed drug pressure (or the ongoing selection of resistant strains as the virus needs to change two nucleotides at the codon 215 position to develop resistance and must go through an intermediate amino acid such as C/D/S before Y or F is selected, e.g. ACC (T) → TCC (S) → TAC (Y)). Development of new resistance comprised all patients with first-time drug-resistant or revertant mutations within the period of the study. Three methods were employed to assess the change in resistance over time within each group of patients as described below. Method 1 is the primary outcome of this study and is the only method for which further statistics were computed.

Method 1: Prevalence of resistance was calculated at baseline as a percentage of patients with resistance out of a sample of all patients with known ND VL status, WT genotype, resistant genotype, or revertant genotype. Due to incomplete genotype data at certain time points for a small number of patients (for certain samples, only a partial

resistance genotype assay was performed due to inadequate plasma sample – see Discussion for further explanation/limitations), sample size varied by time point. In this method, only patients with complete genotype data for all time points comprised the sample. The number of patients with first-time resistance in each subsequent time point was added to the cumulative incidence of resistance. The Mantel-Haenszel chi-square test for linear trends was used to test for significance of changes in cumulative resistance.

Method 2: The incidence of resistance was calculated for each transition between two time points. Patients with either 1) known development of first-time resistance, 2) known continuing resistance, or 3) known continuing ND VL or WT genotype for all time points comprised the total sample. The sample size was higher than in Method 1, as patients with resistant genotypes at any earlier time point within the study would be automatically characterized as known continuing resistance at subsequent time points regardless of the completeness of the data at subsequent time points. Patients not meeting any of the three criteria at any single time point were excluded from the sample at all time points. For example, a patient with ND VL at baseline, WT genotype at 6 months, and incomplete genotype data at 12 months would be excluded from the sample at all transition points. Excluding these patients maintained a constant sample size over all time points.

Method 3: Similarly to Method 2, this method also assessed the new development of resistance as opposed to prevalence of cumulative resistance. Unlike the previous two methods, the sample size varies at each time point. Patients not meeting any of the three criteria at any single time point were only excluded from the sample at that specific time

point. The patient in the Method 2 example would still be included in the sample size at the first transition point between baseline and 6 months as not newly resistant.

Adherence

Of note, collection of data on rates of optimal adherence at each time point began only after the baseline of the study, as it was not part of the initial study. Thus, adherence data is incomplete at all time points. Only a descriptive analysis of adherence and resistance was able to be completed.

RESULTS

Population Characteristics

Resistance data was available for 396 patients from YNHH (n=192) and HH (n=204). Table 1 summarizes characteristics of the study population prior to study initiation. The sample sizes in this table reflect the number of patients that answered the question on the survey.

TABLE 1. Characteristics of Study Population.[A]

		YNHH	HH	TOTAL
DEMOGRAPHICS				
Mean Age (N=390)	**--**	43.7	43.2	**43.4**
Gender (N=395)	**Male**	101 (53%)	123 (60%)	**224 (57%)**
	Female	90 (47%)	81 (40%)	**171 (43%)**
Education (N=394)	**> High School**	69 (36%)	105 (51%)	**174 (47%)**
	HS Diploma	76 (40%)	70 (34%)	**146 (37%)**
	Secondary	45 (24%)	29 (15%)	**74 (16%)**
Annual Income (N=395)	**< $10K**	135 (71%)	148 (73%)	**283 (72%)**
	$10K-$50K	45 (23%)	40 (20%)	**85 (22%)**
	> $50K	11 (6%)	16 (7%)	**27 (6%)**
Welfare (N=395)	**Yes**	119 (62%)	159 (78%)	**278 (70%)**
	No	72 (38%)	45 (22%)	**117 (30%)**
Race (N=389)	**African-American**	96 (51%)	52 (26%)	**148 (38%)**
	Latino	27 (14%)	108 (54%)	**135 (35%)**
	White	56 (30%)	31 (16%)	**87 (22%)**
	Other	10 (5%)	9 (4%)	**19 (5%)**
HIV CHARACTERISTICS				
On HAART therapy	**Yes**	141 (78%)	123 (60%)	**264 (68%**
(N=386)	**No**	41 (23%)	81 (40%)	**122 (32%)**

[A] Data is valid at time of study initiation. N = number of patients for whom data is complete.

Duration of HAART (N=187)	**< 3 years**	50 (43%)	40 (30%)	**90 (48%)**
	>3 years	68 (57%)	93 (70%)	**97 (52%)**
Duration of HIV infection (N=394)	**≤ 2 years**	23 (12%)	23 (12%)	**46 (12%)**
	3-10 years	105 (55%)	106 (56%)	**227 (58%)**
	≥ 10 years	62 (33%)	59 (32%)	**121 (30%)**
HIV Risk Factor (N=387)	**Homosexual**	27 (14%)	14 (7%)	**41 (11%)**
	Heterosexual	86 (46%)	94 (47%)	**180 (47%)**
	IDU	67 (36%)	89 (45%)	**156 (40%)**
	Blood Transfusion	7 (4%)	3 (1%)	**10 (2%)**

Differences in population characteristics between the HH and YNHH patients participating in the resistance substudy existed in the percentage of patients on HAART (60% at HH vs. 78% at YNHH (χ^2 (1, N=386) = 13.131, p = .000)), length of therapy (70% at HH were on HAART for > 3 years vs. 57% at YNHH (χ^2 (1, N=251) = 4.111, p = .042)), education (51% at HH had not graduated high school vs. 36% at YNHH (χ^2 (1, N=251) = 9.162, p = .000)), percentage of patients on welfare (78% at HH vs. 62% at YNHH (χ^2 (1, N=395) = 11.571, p = .001)), and racial composition (54% Hispanic and 26% African-American at HH vs. 14% Hispanic and 51% African-American at YNHH (χ^2 (3, N=389) = 68.661, p = .000)).

Table 2 provides a cross-sectional analysis of genotype at each time point, i.e. baseline, 6 months, 12 months, and 18 months for the entire sample. Sample size varies across time points as samples were tested for resistance from discard plasma only if a viral load was tested for at the clinic visit and enough plasma remained for genotype testing. Because no extra blood was drawn from patients, not every patient had a sample available for genotype testing at every time point. The percentages of patients with ND

VL were 46.6% at baseline, 52.2% at 6 months, 54.4% at 12 months, and 56.7% at 18 months. The percentages of patients with WT genotype were 26% at baseline, 14.2% at 6 months, 17.1% at 12 months, and 21% at 18 months. Overall, 27.4% of patients had drug-resistant mutations at baseline, 33.6% had drug-resistant mutations at 6 months, 28.5% of patients had drug-resistant mutations at 12 months, and 22.3% of patients had drug-resistant mutations at 18 months (this represents a cross-sectional prevalence of resistance – changes cumulative resistance based on historical genotype, the primary outcome of the study, will be presented in the next section). Also see Table 2 for cross-sectional prevalence of resistance by drug class.

TABLE 2. Cross-sectional Prevalence of Genotype in Study Population.

Genotype	Baseline	6 months	12 months	18 months
ND VL	158 (46.6%)	129 (52.2%)	105 (54.4%)	89 (56.7%)
WT	88 (26.0%)	35 (14.2%)	33 (17.1%)	33 (21.0%)
Total Resistant	93 (27.4%)	83 (33.6%)	55 (28.5%)[B]	35 (22.3%)[C]
1-class[D]	51 (55%)	21 (25%)	18 (33%)	21 (60%)
2-class	33 (35%)	33 (40%)	23 (42%)	10 (29%)
3-class	9 (10%)	5 (6%)	2 (4%)	3 (11%)
Revertant	0 (0%)	24 (29%)	10 (18%)	0 (0%)
Patients	**339**	**247**	**193**	**157**

[B] This total includes two samples with resistant mutations on partial resistance genotype assay. Full genotype was not performed.
[C] This total includes one sample with resistant mutations on partial resistance genotype assay. Full genotype was not performed.
[D] Percentages for drug-classes and revertants based on total resistants.

Changes in Resistance

As noted previously, patients were divided into three groups in order to measure changes in the prevalence of cumulative resistance. Out of the total sample of 396 patients, 84 patients had data available for all 18 months of the study (group 1), 153 patients had data available for at least 12 consecutive months (group 2), and 204 patients had data available for at least the first six months from baseline (group 3). Groups are cumulative such that all patients in the 18-month group are included in the 6-month and 12-month groups, and all patients in the 12-month group are included in the 6-month group. Characteristics of patients among these three groups did not greatly differ except for the percentage of patients on HAART therapy. 71% of patients from the 6-month group were on HAART therapy and 69% of patients from the 12-month group, while 83% of patients from the 18-month group were on HAART therapy at the initiation of the study (χ^2 (3, N=386) = 13.794, p = .003). The method of transmission of virus was not different between groups (χ^2 (9, N=387) = 1.816, p = .998). Baseline VL did not significantly differ ($\underline{F}$(3,338) = 2.546, ns).

Tables 3-5 present the cumulative resistance over time within each group of subjects. As noted previously, sample size differs among the different groups. The sample size within each group is also affected by samples with inadequate genomic material (see Discussion for explanation/limitations) for which only a partial resistance genotype assay was performed. These samples were excluded from the analysis. For example, the presence of resistance in a partial genotype assay showing only WT genotype would be unknown. For the 18-month group, 6/84 patients had incomplete

genotype data at 12 months and 18 months. The total sample size is 78 patients with complete data. 3 of these 6 patients at 12 and 18 months had previous drug-resistant mutations with no new resistance possible, giving a sample size for calculation of incidence of new resistance of 81 patients at 12 and 18 months. For the 12-month group, 9/153 patients had incomplete genotype data at 12 months and 6 of these 9 patients had previous drug-resistant mutations again allowing them to be classified as not newly resistant.

Method 1. The prevalence of resistance in the 18-month group (n=78) increased by 14.1% over 18 months from 32.1% to 46.2% (54.2% to 58.3% at HH; 22% to 40.7% at YNHH). Cumulative resistance in the 12-month group (n=144) increased by 18.8% over 12 months from 31.9% to 50.7% (44.4% to 60.3% at HH; 22.2% to 43.2% at YNHH). Cumulative resistance in the 6-month group (n=204) increased by 12.2% from 30.9% to 43.1% (47.2% to 55.1% at HH; 18.3% to 33.9% at YNHH). Figures 2-4 illustrate these changes in cumulative resistance over time within each group of subjects. The Mantel-Haenszel chi-square statistics for linear trends are 1) for the 18-month group (χ^2 (1, n=78) = 3.084, p = .079) 2) for the 12-month group (χ^2 (1, n=144) = 10.328, p = .001. Pearson chi-square was done for the 6-month group (χ^2 (1, n=204) = 6.571, p = .010).

Method 2. The 6-month incidence of resistance in the 18-month group was 7.1% at 6 months (n=84), 1.2% at 12 months (n=81), and 4.8% at 18 months (n=81) (3.4%, 3.6%, 0% at HH; 9.1%, 0%, 7.4% at YNHH). The 6-month incidence of new resistance in the 12-month group was 12.4% at 6 months (n=153) and 5.3% at 12 months (n=150) (10.3%, 4.6% at HH; 14.1%, 5.9% at YNHH). The incidence of new resistance in 6-month group (n=204) was 12.3% at 6 months (7.9% at HH, 15.7% at YNHH).

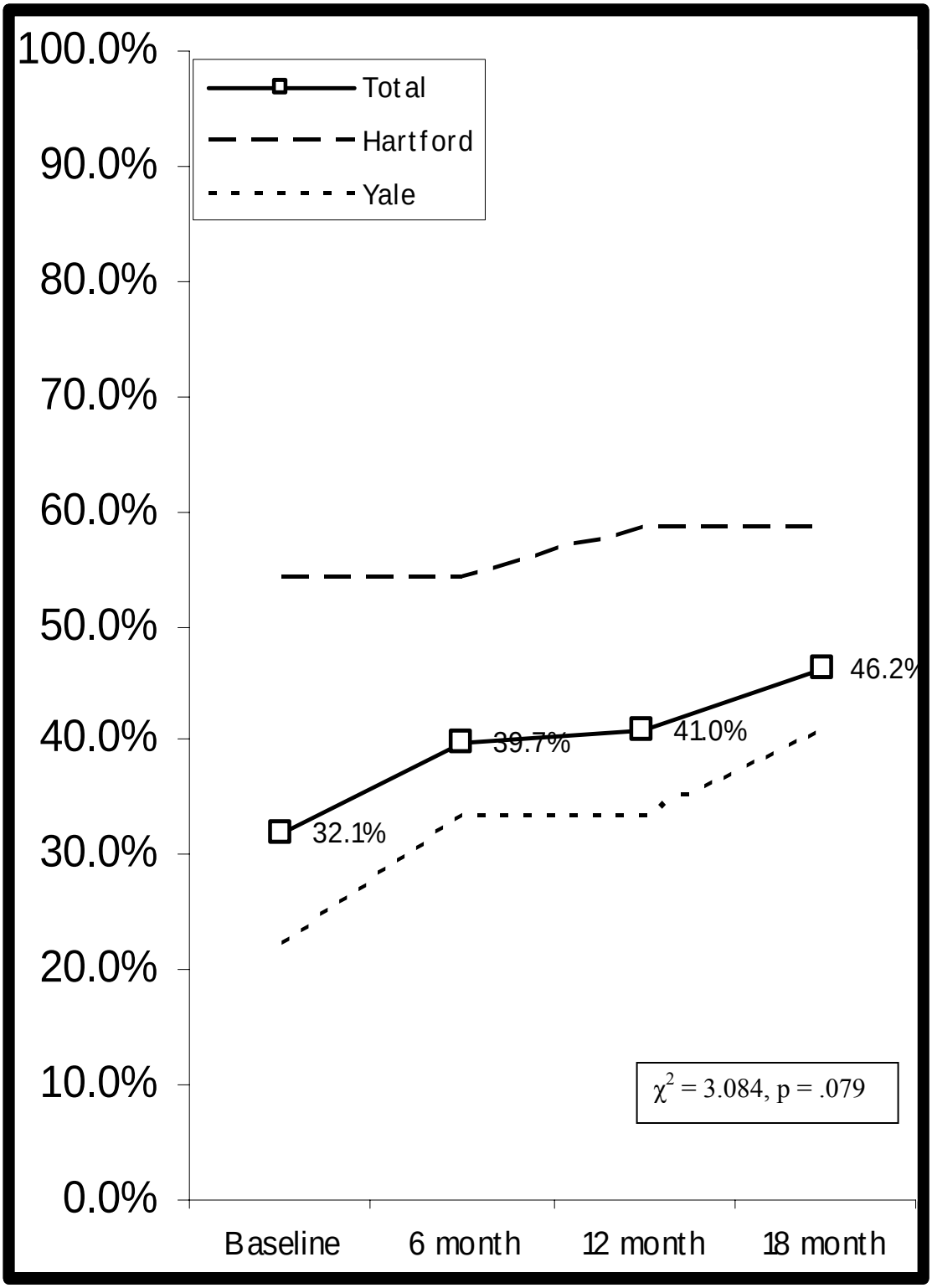
Method 3. The 6-month incidence of resistance in the 18-month group (n=81) was 7.4% at 6 months, 1.2% at 12 months, and 4.9% at 18 months (0%, 3.7%, 0% at HH; 11.1%, 0%, 7.4% at YNHH). The 6-month incidence of new resistance in the 12-month group (n=150) was 12.7% at 6 months and 5.3% at 12 months (10.8%, 4.6% at HH; 14.1%, 5.9% at YNHH). The incidence of new resistance in the 6-month group (n=204) was 12.3% at 6 months (6.8% at HH; 14.8% at YNHH).

TABLE 3. Cumulative resistance and incidence of new resistance among patients with 18 months of genotype data (group 1).

Genotype	Baseline	6 months	12 months	18 months
ND VL	44 (52.4%)	50 (59.5%)	50 (64.1%)	47 (60.3%)
WT	15 (17.9%)	10 (11.9%)	9 (11.5%)	11 (14.1%)
Total Resistant	25 (29.8%)	24 (28.6%)	19 (24.4%)	20 (25.6%)
1-class[E]	13 (52%)	4 (17%)	6 (32%)	13 (65%)
2-class	9 (36%)	16 (67%)	11 (58%)	6 (30%)
3-class	3 (12%)	2 (8%)	1 (5%)	1 (5%)
Revertant	0 (0%)	2 (8%)	1 (5%)	0 (0%)
New Resistance (n=81)	--	**6 (7.4%)**	**1 (1.2%)**	**4 (4.9%)**
Cumulative Resistance (n=78)	**25 (32.1%)**	**31 (39.7%)**	**32 (41.0%)**	**36 (46.2%)**

[E] Percentages for drug-classes and revertants based on total resistants.

FIGURE 2. Prevalence of ART resistance over time in patients with 18 consecutive months of complete genotype data (N=78).[F]



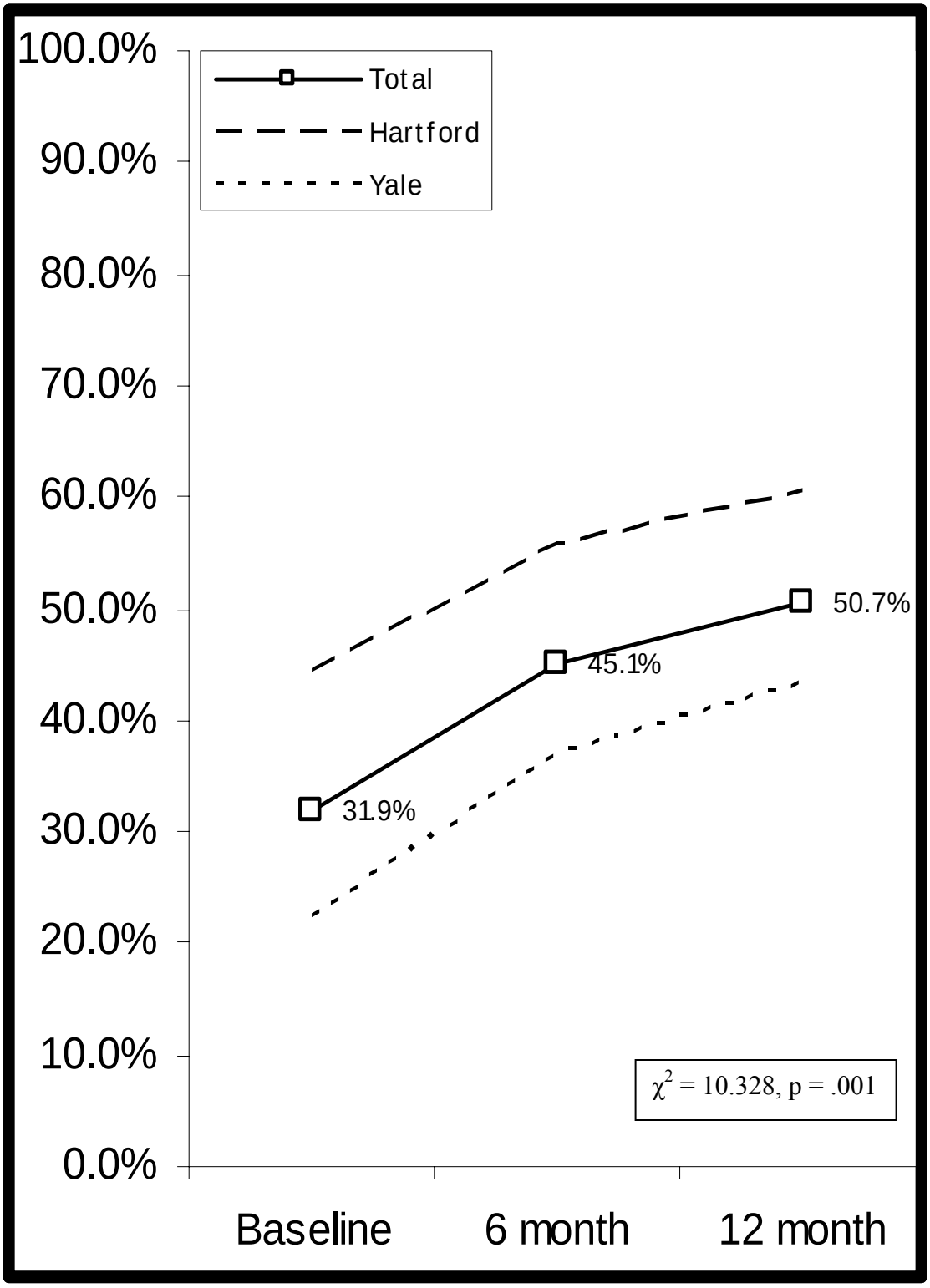
[F] Mantel-Haenzel χ^2 test for linear trends computed for total sample size.

TABLE 4. Cumulative resistance and incidence of new resistance among patients with 12 consecutive months of genotype data (group 2).

Genotype	Baseline	6 months	12 months
ND VL	74 (48.4%)	85 (55.6%)	84 (58.3%)
WT	33 (21.6%)	16 (10.5%)	22 (15.3%)
Total Resistant	46 (30.1%)	52 (34.0%)	38 (26.4%)
1-class[G]	22 (48%)	12 (23%)	13 (34%)
2-class	16 (35%)	22 (42%)	16 (42%)
3-class	8 (17%)	5 (10%)	1 (3%)
Revertant	0 (0%)	13 (25%)	6 (16%)
New Resistance (n=150)	--	**19 (12.7%)**	**8 (5.3%)**
Cumulative Resistance (n=144)	**46 (31.9%)**	**65 (45.1%)**	**73 (50.7%)**

[G] Percentages for drug-classes and revertants based on total resistants.

FIGURE 3. Prevalence of ART resistance over time in patients with 12 consecutive months of complete genotype data (N=144).[H]



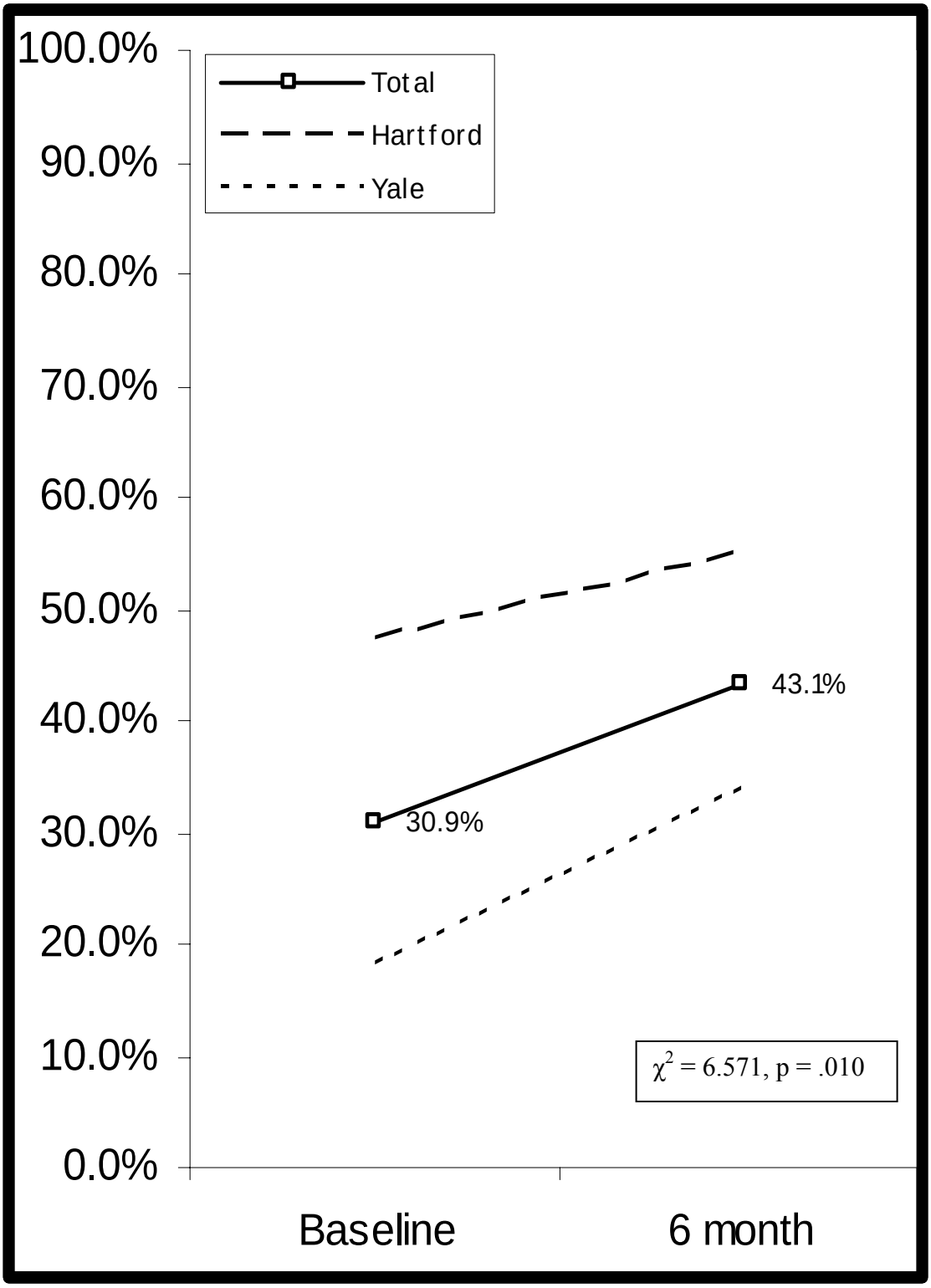
[H] Mantel-Haenzel χ^2 test for linear trends computed for total sample size.

TABLE 5. Cumulative resistance and incidence of new resistance among patients with genotype data at baseline and 6 months (group 3).

Genotype	Baseline	6 months
ND VL	95 (46.6%)	110 (53.9%)
WT	46 (22.5%)	24 (11.8%)
Total Resistant	63 (30.9%)	70 (34.3%)
1-class[1]	32 (51%)	17 (24%)
2-class	23 (37%)	29 (41%)
3-class	8 (12%)	5 (7%)
Revertant	0	19 (28%)
New Resistance (n=204)	--	**25 (12.3%)**
Cumulative Resistance (n=204)	**63 (30.9%)**	**88 (43.1%)**

[1] Percentages for drug-classes and revertants based on total resistants.

FIGURE 4. Prevalence of ART resistance over time in patients with 6 consecutive months of complete genotype data starting at baseline. (N=204).[J]



[J] Pearson χ^2 test computed for total sample.

The 14.1% rate of increase in the 18-month group (mean = .78% per month) was lower than rates of increase in the 12-month group (mean = 1.56% per month) and in the 6-month group (mean = 2.04% per month). The difference in 6-month rates of increase between the three groups was significant ($\chi 2$ (2, N=204) = 7.405, p = .025).

Overall, 37 patients developed new identifiable drug-resistant mutations during the time period of this study. The only significant differences between this group with new resistance and the total sample were the gender and clinic location of the patients. 8/37 patients with new resistance were male while 224/395 (refer to Table 1 for complete statistics) patients were male in the total sample ($\chi 2$ (1, N=395) = 20.472, p = .000). 27/37 patients with new mutations were at YNHH while 10/37 patients were at YNHH ($\chi 2$ (1, N=396) = 9.908, p = .002).

Adherence

Data on the number of patients who maintained optimal adherence (>95% adherence) was available for a small sample of patients at each time point. The table below shows the number of patients optimally adherent at each time point.

Table 6. Number of patients with >95% adherence at each time point

	Baseline	**6 months**	**12 months**	**18 months**
>95% Adherence	55 (72%)	126 (82%)	142 (82%)	122 (82%)
Total[K]	76	154	174	149

[K] Total indicates number of patients for whom this data is available.

A description of the adherence data is provided here due to the smaller sample size for this data. Adherence rates in each group are shown in Table 7.

Table 7. Percentage of patients with >95% adherence rates by group.

	Baseline	6 months	12 months	18 months
6-month group	73% (N=48)[L]	86% (N=106)	--	--
12-month group	63% (N=30)	89% (N=74)	80% (N=104)	--
18-month group	69% (N=13)	88% (N=42)	77% (N=61)	84% (N=61)

Adherence rates among the patients with new development of resistance who had adherence data available during the course of the study were 86% in the first 6 months, 80% in the second 6 months, 80% in the third 6 months, and 63% in the last 6 months. Of patients in the 6-month group who maintained optimal adherence during the first 6 months, 2/18 developed new resistance. Of patients in the 12-month group who maintained optimal adherence in the first 6 months, 2/10 developed new resistance, and 2/49 patients who maintained optimal adherence in the second 6 months developed new resistance. No patients who maintained optimal adherence for at least 6 consecutive months in the 18-month group developed new resistance during the maintenance periods.

Genotype Transitions

Table 8 shows the patterns of changes in genotype for all 6-month transitions in the study. Patients with more than six months of genotype data are thus represented more than once in the sample of all such transitions. This data provides a more complete

[L] N = number of patients for whom data is available.

picture of the complexities of the development of non-detectability, WT genotype, and resistance in a clinic scenario.

TABLE 8. Percentage of genotypes at time point(x) that transition to genotype at time point(x+1).[M]

Genotype at Time Point (x)	Genotype at Time Point (x+1)	Percentage of All Transitions
ND	ND	87.3%
	WT	2.5%
	R	10.2%
WT	ND	25.3%
	WT	53.2%
	R	21.5%
R	ND	28.6%
	WT	10.7%
	R	60.7%

Revertants

The results of an analysis of changes in the prevalence of resistance (using Method 1 from above) both 1) excluding all samples with revertant genotypes and 2) assuming all revertant genotypes as not resistant are shown in Table 9 and Table 10 below respectively.

[M] ND = nondetectable; WT = wild-type; R = drug-resistant

Table 9. Cumulative resistance (using Method 1) for all three groups excluding revertants from analysis.

Group	Baseline	6 months	12 months	18 months
6-month (n=185)	63 (34.1%)	77 (41.6%)	--	--
12-month (n=127)	46 (36.2%)	56 (44.1%)	72 (56.7%)	--
18-month (n=75)	25 (33.3%)	31 (41.3%)	31 (41.3%)	36 (48.0%)

Table 10. Cumulative resistance (using Method 1) for all three groups assuming revertant genotypes are not resistant.

Group	Baseline	6 months	12 months	18 months
6-month (n=204)	63 (30.8%)	77 (37.8%)	--	--
12-month (n=144)	46 (31.9%)	56 (38.9%)	72 (50.0%)	--
18-month (n=78)	25 (32.1%)	31 (39.7%)	31 (39.7%)	36 (46.1%)

Overall, rates of increase in cumulative resistance were similar with and without inclusion of revertants for the 6-month group (χ^2 (1, N=204) = 2.799, p =. 064) and 12-month group (χ^2 (1, N=144) = .023, p =. 879), and rates of increase were identical for the 18-month group. The chi-square tests for significance were as follows: 1) 18-month group (χ^2 (1, n=78) = 3.142, p = .076) 2) 12-month group (χ^2 (1, n=78) = 9.736, p = .002) 3) 6-month group (χ^2 (1, n=78) = 2.131, p = .144).

Regarding transitions involving revertant genotypes, of 34 total samples with revertant genotypes, 14 samples had genotype status available for the subsequent time point, and 25 samples had genotype status available for the preceding time point. Of the 14 revertant samples with subsequent time point genotype available, 5 transitioned to

ND, 6 transitioned to WT genotype, 1 transitioned to resistant genotype, and 2 remained revertant. Of the 25 revertant samples with preceding time point genotype available, 3 transitioned to revertant status from ND, 9 transitioned from WT genotype, 11 transitioned from resistant genotype, and 2 transitioned from revertant genotype. Of note, because patients went on and off ART throughout the study (and especially between 6-month genotyping time points) due to variation in adherence, patient choice, and clinician-driven treatment interruptions, a minority of these transitions involve transitions to or from WT genotype.

DISCUSSION

In this study, we analyzed patterns of ART resistance in a sample of HIV+ patients from the two largest HIV clinics in Connecticut and examined changes in prevalence of resistance utilizing longitudinal genotype testing. This study provides an insight into the dynamics of how antiretroviral resistance changes over time in a population of HIV+ patients under a typical clinical care setting. We found a 14.1% increase in resistance among patients with 18 months of genotype data, an 18.8% increase among patients with 12 months of genotype data, and a 12.2% increase among patients with 6 months of genotype data. These findings demonstrate that the burden of resistance in clinical populations is great and a major issue in the care of patients (from the time period of 2000-2003).

The hypothesis in this study was that the cumulative prevalence of ART resistance in this patient population would increase at a rate of >5% per 6-month interval and the cumulative rate of increase during the 18-month period of data in this study will exceed 15%. Prevalence of resistance increased in patients who had genotype data available for 6 months, 12 months, and 18 months. The 14.1% increase in cumulative resistance in the 18-month population did not meet the hypothesized 15% increase in prevalence over 18 months. As few studies have examined historical genotypes, the hypothesis set at the beginning of this study was only an estimate. The results in this study approached the hypothesized 15% increase and have equally important consequences. Also, the 6-month increase in cumulative resistance was greater than 5% in the 6-month group, both 6-month periods in the 12-month group, and the first and last 6-month period of the 18-month group. Observing the incidence of new resistance, this

was greater than 5% in all 6-month periods except for the second and third 6-month period of the 18-month group.

The 18-month group had the lowest rate of increase in the cumulative resistance. It is possible that patients who made all four clinic visits over 18 months (and thus were more likely to come to clinic and keep appointments) led more stable lives with less active drug use and were seen more frequently by their clinician, which may have provided more opportunity to change therapy sooner before resistance emerged. These data were not captured in the present study. Appointment nonadherence has been associated with failure to suppress viral load at two or more time points and with AIDS-defining CD4 counts (36, 37), although Purkayastha *et al.* did not find an association between clinic appointments and ND VL (38). The 18-month group may have had higher rates of viral suppression during the study period. Several studies have also associated lower rates of adherence with missed clinical appointments (39, 40). The available data does not show greater rates of adherence to drug therapy in this 18-month group. However, this was difficult to assess statistically in the present study due to incomplete data on adherence. As discussed in the introduction, a higher level of adherence (>95%) to antiretroviral therapy with viral suppression is linked to lower levels of development of drug resistance. Sub-optimal adherence to certain classes of drugs may lead to higher levels of resistance (16, 17). More data on average rates of adherence would be useful to characterize these relationships within the groups in this study. IDU and high baseline plasma VL are also associated with development of resistance. The groups did not differ significantly in IDU use as a risk factor for original HIV infection or in mean plasma VL.

Cumulative resistance increased at both clinical sites in the study. The rate of development of new resistance at YNHH was greater than at HH. Since HH had a higher initial prevalence, the population may have been closer to the "plateau" of resistance. At 54%, the baseline resistance at HH was 2.5 times the baseline resistance at YNHH of 22%. At such high levels of baseline resistance, it is possible that this plateau of resistance was reached in this inner city community clinic, which could account for the lower rate of new resistance development at HH. As very high levels of adherence are associated with a decreased likelihood of developing drug resistance, a plateau effect may be possible if a core subset of the population maintains excellent adherence. Further, a portion of the remaining non-resistant patients may not be on drug therapy and thus have no selective pressure for resistance. One could hypothesize that the rate of resistance in any population would reach a plateau once the major at-risk populations have developed resistance (e.g. poor adherers and patients on insufficiently potent regimens). Alternately, the difference in baseline levels of resistance and development of new resistance may reflect differences in provider skills, prescribing habits, demographics, and adherence. The HH clinic population had more Hispanic patients, lower levels of education, and a higher percentage of patients on welfare. A longer period of genotypic testing is needed to determine whether a plateau effect exists and predictors of such an effect. Other clinic populations should be studied as well. Of note, as discussed earlier, the population of chronically-infected HIV patients studied by Richman *et al.* between 1996 and 1999 showed 48% resistance. This is similar to the resistance rates at HH documented for the period immediately following from 2000-2003. The HH clinic population may represent

a similar group to this early HAART era population. It is possible that a prevalence of 50% represents a resistance level approaching a plateau.

Prior studies did not genotype all available samples at all time points and thus may have missed some resistance. This study represents a more in-depth look at trends in resistance than the Harrigan *et al*. study (23), which originally advocated the longitudinal testing approach based on historical genotype as opposed to the cross-sectional genotyping method for following changes in resistance. That study represented ~29% of treated HIV-1 infected individuals in British Columbia. In the Harrigan *et al*. study, genotype testing was performed at the discretion of the clinician and was not done for all samples with detectable VL as in the present study. The frequency of genotyping was a median of five tests over a median of 34 months. The frequency of genotype testing in our study was four tests over an 18 month period. While our study looked at all patients under clinical care participating in the resistance substudy, the Harrigan *et al*. study was biased towards patients in whom therapy was failing and for whom clinicians saw value in performing multiple tests for drug resistance.

By identifying specific mutations, genotypic assays have become a common method in the detection of drug resistance. However, there are a few limitations that arise from this method. As with any laboratory testing methods, there is the potential for laboratory error in the methods as well as reporting of results. Of note, genotype assays may not be able to detect mixtures of wild-type and resistant viral populations, as current assays can only detect viruses representing greater than 5 to 20 percent of the plasma viral population (12). Due to the possibility of latent strains of resistant virus, resistance may go undetected in certain individuals at certain time points as they test only virus

circulating in plasma. As the present study performed genotypes on longitudinally obtained samples thus obtaining both historical and current genotypes to characterize the prevalence of resistance, this limitation was offset to some degree. To fully correct this limitation, sampling of the archived virus from latently infected T-cells and sampling of sanctuary sites such as the genital-urinary compartment and central nervous system would need to be performed.

The genotype testing in our study was limited to available samples from discard plasma originally tested for VL. Complete genotyping was not possible on a few samples because 1) a genotype was attempted on all samples irrespective of viral load (most clinical labs will not test a sample for resistance if the viral load is < 1000 copies as the yield is low and genetic material is insufficient for sequencing 2) no additional blood was drawn other than that for routine clinical care; this discard plasma was freezed-thawed at least twice, which can lead to decreased viral RNA copies.

Patients in this study represent a diverse population of patients with regards to HIV risk factors, socioeconomic and racial background, and sexual orientation. Of patients with such data available, 68% were already on HAART therapy prior to baseline, 52% had been on HAART therapy for greater than three years, and 88% had been infected with HIV for greater than two years. While this clinical setting provides a realistic look at development of resistance for clinicians, certain limitations arise in this study. The time course of HIV infection and HAART therapy factoring into baseline genotype is unique for each patient. The presence of back-revertants in a minority of patients likely reflects the fact that patients frequently went on and off ART, as is real-life clinical situations. In the main analysis, revertant genotypes were counted as drug-

resistant. From the analysis of changes in cumulative resistance both excluding revertants and assuming revertants are not resistant, because patients' genotypes prior to baseline are unknown, overall prevalence of resistance is likely underestimated. ND VL at baseline does not necessarily indicate absence of resistance prior to baseline. Thus, while this study indeed relies on historical genotype to calculate cumulative resistance, the baseline level of resistance represents a cross-sectional resistance at the arbitrary point in time of enrollment in the study. Furthermore, enrollment in the Options Project was staggered over two years. The data on cross-sectional resistance presented in Table 2 provides information relative to each patient's baseline, and cannot be interpreted as a trend beginning in a specific year.

Further studies are needed to examine the possibility of a resistance plateau as discussed earlier. Also, while this study only examined development of first-time resistance in clinic populations, a further analysis of patterns of the accumulation of additional drug-resistant mutations would be useful in the clinical setting.

Antiretroviral resistance will remain as a major challenge as increasing numbers of HIV+ patients are placed on treatment and transmission of drug-resistant virus continues in the community. The prevalence of antiretroviral resistance in all groups of HIV+ patients has been increasing over the past decade with the advent of multi-drug HAART regimens. This study is among the first to look at these patterns of antiretroviral resistance in a typical HIV+ patient population under clinical care. Prevalence of resistance in this community has risen dramatically. The findings of this study demonstrate the importance of monitoring for HIV drug resistance trends in patients in

clinical care (as is done routinely for other infectious pathogens, e.g. hospital and community acquired infections). Further, these data can help in the design of future studies on how best to address resistance in a clinical setting through physician education, treatment regimen adjustments, reduction of transmission risks, and utilization of genotype testing.

REFERENCES

1. UNAIDS/WHO. AIDS Epidemic Update UNAIDS/05.19E. December 2005. Accessed January 15, 2006. < http://www.unaids.org/Epi2005/doc/EPIupdate2005_pdf_en/epi-update2005_en.pdf>.

2. Glynn M, Rhodes P. Estimated HIV prevalence in the United States at the end of 2003. 2005. National HIV Prevention Conference, Atlanta. (Abstr. 595).

3. Rothenberg R, Woelfel M, Stoneburner R, Milberg J, Parker R, Truman B. Survival with the acquired immunodeficiency syndrome. Experience with 5833 cases in new york city. N Engl J Med. 1987; 317(21):1297-1302.

4. Larder BA, Darby G, Richman DD. HIV with reduced sensitivity to zidovudine (AZT) isolated during prolonged therapy. Science. 1989; 243(4899):1731-4.

5. Kozal MJ, Shafer R, Winters M, Katzenstein D, Merigan TC. A mutation in human immunodeficiency virus reverse transcriptase and decline in CD4 lymphocyte numbers in long-term zidovudine recipients. JID. 1993; 167(3):526-32.

6. Kozal MJ, Kroodsma K, Winters MA, Katzenstein DA, Halpern J, et al. Development of a didanosine (ddI) resistance mutation in 64 HIV-infected patients switched from zidovudine (ZDV) to ddI monotherapy: relationship to CD4+ T cell changes and virus burden. Ann Int Med. 1994; 121:253-268.

7. Pomerantz RJ, Horn DL. Twenty years of therapy for HIV-1 infection. Nat Med. 2003; 9(7):867-873.

8. Erice A, Mayers DL, Strike DG, Sannerud KJ, McCutchan FE, et al. Brief report: primary infection with zidovudine–resistant human immunodeficiency virus type 1. N. Engl. J. Med. 1993; 328(16):1163-5.

9. Ledergerber B, Egger M, Opravil M, et al. Clinical progression and virological failure on highly active antiretroviral therapy in HIV-1 patients: A prospective cohort study. swiss HIV cohort study. Lancet. 1999; 353(9156):863-868.

10. Wainberg MA, Friedland G. Public health implications of antiretroviral therapy and HIV drug resistance. JAMA. 1998; 279(24):1977-1983.

11. Deeks SG. Treatment of antiretroviral-drug-resistant HIV-1 infection. Lancet. 2003; 362(9400):2002-2011.

12. Daar ES. Antiretroviral resistance in clinical practice. J Int Assoc Physicians AIDS Care (Chic Ill). 2003; 2 Suppl 1:S4-18.

13. Kroodsma KL, Kozal MJ, Hamed KA, Winters MA, Merigan TC. Detection of drug resistance mutations in the human immunodeficiency virus type 1 (HIV-1) pol gene: differences in semen and blood HIV-1 RNA and proviral DNA. J Infect Dis. 1994; 170(5):1292-5.

14. Clavel F, Hance AJ. HIV drug resistance. N Engl J Med. 2004; 350(10):1023-1035.

15. Harrigan PR, Hogg RS, Dong WW, et al. Predictors of HIV drug-resistance mutations in a large antiretroviral-naive cohort initiating triple antiretroviral therapy. J Infect Dis. 2005; 191(3):339-347.

16. Bangsberg DR. Porco TC. Kagay C. Charlebois ED. Deeks SG. Guzman D. Clark R. Moss A. Modeling the HIV protease inhibitor adherence-resistance curve by use of empirically derived estimates. JID. 2004; 190(1):162-5.

17. Bangsberg DR. Moss AR. Deeks SG. Paradoxes of adherence and drug resistance to HIV antiretroviral therapy. J Antimicrob Chemother. 2004; 253(5):696-9.

18. Little SJ, Daar ES, D'Aquila RT, et al. Reduced antiretroviral drug susceptibility among patients with primary HIV infection. JAMA. 1999; 282(12):1142-1149.

19. Weinstock HS. Zaidi I. Heneine W. Bennett D. Garcia-Lerma JG. Douglas JM Jr. LaLota M. Dickinson G. Schwarcz S. Torian L. Wendell D. Paul S. Goza GA. Ruiz J. Boyett B. Kaplan JE. The epidemiology of antiretroviral drug resistance among drug-naive HIV-1-infected persons in 10 US cities. JID. 2004; 189(12):2174-80.

20. Bennett D, et al. 12th CROI. Boston, 2005. Abstr. 674.

21. Novak RM, Chen L, MacArthur RD, et al. Prevalence of antiretroviral drug resistance mutations in chronically HIV-infected, treatment-naive patients: Implications for routine resistance screening before initiation of antiretroviral therapy. Clin Infect Dis. 2005; 40(3):468-474.

22. Richman DD, Morton SC, Wrin T, et al. The prevalence of antiretroviral drug resistance in the united states. AIDS. 2004; 18(10):1393-1401.

23. Harrigan PR, Wynhoven B, Brumme ZL, et al. HIV-1 drug resistance: Degree of underestimation by a cross-sectional versus a longitudinal testing approach. JID. 2005; 191(8):1325-1330.

24. Lundgren JD et al. Risk of death following triple class virological failure: The Plato Collaboration. 43rd ICAAC, San Diego, September 2003. Abstr. H-450.

25. Little SJ, Holte S, Routy JP, et al. Antiretroviral-drug resistance among patients recently infected with HIV. N Engl J Med. 2002; 347(6):385-394.

26. Grant RM, Hecht FM, Warmerdam M, et al. Time trends in primary HIV-1 drug resistance among recently infected persons. JAMA. 2002; 288(2):181-188.

27. Miller V, Phillips A, Rottmann C, Staszewski S, Pauwels R, et al. Dual resistance to zidovudine and lamivudine in patients treated with zidovudine-lamivudine combination therapy: associated with therapy failure. JID. 1998; 177:1521-32.

28. Hogg RS, et al. 12th CROI. Boston, 2005. Abstr. 712.

29. D'Aquila RT, Johnson VA, Welles SL, Japour AJ, Kuritzkes DR, et al. Zidovudine resistance and HIV-1 disease progression during antiretroviral therapy. Ann Int Med. 1995; 22(6):401-408.

30. Katzenstein DA, Bosch RJ, Hellmann N, Wang N, Lee B, et al. Phenotypic susceptibility and virological outcome in nucleoside-experienced patients receiving three or four antiretroviral drugs. AIDS. 2003; 17(6):821-830.

31. Kozal MJ, Amico KR, Chiarella J, Cornman D, Fisher W, et al. HIV drug resistance and HIV transmission risk behaviors among active injection drug users. JAIDS. 2005; 40(1):106-9.

32. Kozal MJ, Amico KR, Chiarella J, Schreibman T, Cornman D, et al. Antiretroviral resistance and high-risk transmission behavior among HIV-positive patients in clinical care. AIDS. 2004; 18(16):2185-9.

33. Fisher JD, Fisher WA, Cornman DH, Amico RK, Bryan A et al. Clinician-Delivered Intervention During Routine Clinical Care Reduces Unprotected Sexual Behavior Among HIV-Infected Patients. JAIDS. 2006; 41(1):44-52.

34. Kozal MJ, Shah N, Shen N, Yang R, Fucini R, et al. Extensive polymorphisms observed in HIV-1 clade B protease gene using high-density Oligonucleotide arrays. Nat Med. 1996; 2(7):753-9.

35. Johnson VA, Brun-Vezinet F, Clotet B, et al. Update of the drug resistance mutations in HIV-1: 2004. Top HIV Med. 2004; 12(4):119-24.

36. Lucas GM. Chaisson RE. Moore RD. Highly active antiretroviral therapy in a large urban clinic: risk factors for virologic failure and adverse drug reactions. Ann Int Med. 1999; 131(2):81-7.

37. Berg MB, Safren SA, Mimiaga MJ, Grasso C, Boswell S et al. Nonadherence to medical appointments is associated with increased plasma HIV RNA and decreased CD4 cell counts in a community-based HIV primary care clinic. AIDS Care. 2005; 17(7):902-907.

38. Purkaystha T, Wasi F, Shuter J. Factors Associated with Sustained Virologic Suppression in Patients Receiving Antiretroviral Therapy in an Urban HIV Care Clinic. AIDS Pat Care Stds. 2005; 19(12):785-793.

39. Fong OW. Ho CF. Fung LY. Lee FK. Tse WH. Yuen CY. Sin KP. Wong KH. Determinants of adherence to highly active antiretroviral therapy (HAART) in Chinese HIV/AIDS patients. HIV Med. 2003; 4(2):133-8.

40. Wagner G. Placebo practice trials: the best predictor of adherence readiness for HAART among drug users? HIV Clin Trials. 2003; 4(4):269-81.