INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

Bell & Howell Information and Learning
300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA
800-521-0600

Université d'Ottawa · University of Ottawa

The Association Between Surrogate Marker Response Measures and the Development of Opportunistic Illnesses in HIV-Infected Persons Enrolled in a Large Randomized Clinical Trial

by

Stephen Kravcik MD FRCPC
Division of General Medicine
Ottawa Hospital, General Campus

Thesis submitted to the School of Graduate Studies and Research
in fulfilment of the requirements
for the Master of Science Degree in Epidemiology

University of Ottawa

November 20, 1999

Acknowledgements

Supervisors: Rama C. Nair, MStat, PhD, FACE

Professor, Epidemiology and Community Medicine

University of Ottawa

D. William Cameron MD FRCPC

Division of Infectious Diseases

Ottawa Hospital, General Campus

My thanks to Abbott Laboratories (Abbott Park, Illinois) for the use of the database from M94-247 for this thesis.

Table of Contents

List of Tables

List of Figures and Illustrations

Glossary

ACTG: - AIDS Clinical Trials Group, an American, government-sponsored, multi-centre group of investigators for HIV research

AIDS: - acquired immunodeficiency syndrome; the name applied to HIV infection once a major opportunistic illness has occurred

antigen: - a molecule that stimulates an immune response

antiretroviral therapy: - medical therapy directed at the suppression of HIV replication

ARC: - AIDS-related complex; an outdated term for the symptomatic phase of HIV infection occurring before the onset of a major opportunistic illness

bDNA: - branch chain DNA assay; a method by which RNA or DNA is quantitated

CD4 T lymphocyte: - a lymphocyte, also known as a T helper cell, generally responsible for triggering immune responses to foreign antigens. Selectively depleted by HIV.

genome: - the entirety of genetic information for an organism

gp41: - glycoprotein 41; a component of a major HIV surface membrane protein

HIV-1: - human immunodeficiency virus type 1

IgA: - immunoglobulin A; a type of antibody often found on mucosal surfaces

IL-2: - interleukin 2; a cytokine involved in cell to cell communication

lymphocyte: - a type of white blood cell involved in specific immune responses

non-nucleoside reverse transcriptase inhibitor: - also known as an NNRTI; a drug that non-competitively inhibits HIV reverse transcriptase inhibitor

opportunistic illness (OI): - an illness occurring as a result of immune suppression

major opportunistic illness: - one of 20 potentially severe opportunistic illnesses typical

of HIV infection

minor opportunistic illness: - one of many possible mild illnesses occurring in HIV infection

p24 antigen: - an HIV membrane protein

PCR: - polymerase chain reaction; a method by which RNA or DNA is quantitated

protease inhibitor: - also known as a PI; drugs that inhibit HIV protease, which is required for post-translational cutting of HIV proteins

reverse transcriptase: - the viral enzyme responsible for transcribing HIV's RNA into DNA, which is then integrated into the CD4 cell genome

nucleoside reverse transcriptase inhibitor: - also known as an RTI (in this study) or nRTI; drugs that competitively inhibit reverse transcriptase

surrogate marker: - a lab test, or otherwise, used to monitor therapy in the absence of a method to directly monitor the clinical effectiveness of therapy

transcription: - the transformation of RNA into DNA, or vice versa

translation: - the formation of protein from an mRNA template

viral load: - the number of viruses, measured in genome copies per millilitre

virion: - a single virus

Abstract

Introduction

Surrogate marker responses are imperfect indicators of response to antiretroviral therapy in HIV. It is proposed that the area under the curve of surrogate marker response will be superior to peak response, or to that measured after a period of therapy.

Methods

The database from a study of ritonavir in advanced HIV was used. Using logistic regression, the specificity of the surrogate marker level at baseline, change between baseline and at time points to week 16, peak response, and area under the curve of the response to week 16 and week 40 were determined. The predictive values, likelihood ratios and receiver operating characteristic curves were determined for those of highest specificity.

Results

Specificities increased from baseline to week 16. Peak responses were inferior to time period surrogate marker changes, whereas the areas under the curve were comparable or better than the time period surrogate marker changes. The highest specificity at any time point was for the CD4 change at week 8 (55.90%), whereas the highest overall specificity was for the 16 week AUC for the CD4% (69.63%). However, the PPV, NPV, likelihood ratios and ROC curves demonstrated poor performance overall for these surrogate markers. Too few subjects had viral load testing for this marker to be assessed.

Discussion

Within the limits of this study, it was demonstrated that the CD4 and CD4% were the surrogate markers most associated with clinical outcome, with the CD4% AUC to 16 weeks having the highest overall specificity and the week 8 CD4 having the greatest specificity for clinical use. However, all surrogate markers had specificities below 70%.

1. Introduction

1.1. Background

The term "AIDS" refers to HIV infection that has been complicated by opportunistic (immune deficiency-related) illness (Appendix 1). The first cases were reported in 1981[1]; since then, AIDS has become a significant public health problem[2]. From 1981 to 1990, HIV-related illnesses were responsible for 101,000 deaths in the United States[3], and in 1994 alone, 41,930 U.S. residents died as the result of HIV infection[4]. There were 15,935 reported AIDS cases in Canada to June 30, 1998, with 11,381 reported deaths[5]. It is estimated that there are some 30-40,000 HIV-infected persons in Canada[5].

HIV selectively infects and kills cells with CD4 T lymphocytes (herein referred to as "CD4 cell"), a cell vital to the normal function of the immune system. HIV-mediated CD4 cell death gradually leads to a progressive destruction of the immune system, allowing the development of opportunistic illnesses and death. This is a chronic process, with the time from infection to clinical illness or death measured in years. Studies of homosexual men infected by HIV in the early 1980's demonstrated that the median time from initial infection to death was 10 years[6]. By 13 years after infection, 70% of infected men had died, and 20% had had an AIDS-defining illness. However, with the use of antiretroviral therapy and specific prophylaxis for opportunistic infections, the natural history of HIV infection, and causes of death, have changed[7,8,9]. In general, HIV-infected persons are living longer and the death rate as declined significantly.

1.2. Antiretroviral Therapy, Surrogate Markers and Clinical Illness

It has been demonstrated that HIV replication occurs at very high rates throughout the course of HIV infection, with the production of some 10^9 to 10^{11} virions daily. It has been calculated that a similar number of CD4 T lymphocytes are generated on a daily basis[10]. As antiretroviral drugs inhibit HIV

replication, and HIV kills CD4 cells, these drugs also prevent CD4 cell death. This is manifested as an increase, of variable magnitude and duration, in the number of CD4 cells in the blood after the initiation of therapy. The improved immune system results in protection from opportunistic illnesses and prolonged survival. Treatment of HIV infection with antiretroviral drugs is therefore targeted at viral replication, not symptoms; treatment rarely leads to immediate improvement in symptoms or sense of well being.

The plasma HIV RNA level, representing individual HIV virions, commonly referred to as the "viral load", is measured as copies/millilitre (or $\log_{10}$ copies/mL) of blood plasma. Although highly variable for any level of CD4 cell count, the viral load for any individual tends to remain relatively constant over short periods of time (six to twelve months)[11,12], slowly increasing over longer periods.

Response to antiretroviral therapy has typically been measured in two ways: the reduction in viral load from baseline as measured in log units, or increase in CD4 cell count from baseline. Of less defined usefulness are the CD4%, CD8 cell count (another type of lymphocyte) and CD8%. In clinical practice and research, the responses in CD8 and CD8% have been poorly described and rarely used to assess antiretroviral effectiveness. The CD4% has been considered a marker of lesser variability than the absolute CD4.

The antiretroviral drugs in general use in Canada are of three classes. The first two, the nucleoside analogue reverse transcriptase inhibitors ("RTIs") and non-nucleoside reverse transcriptase inhibitors ("NNRTIs"), work to inhibit reverse transcriptase, the HIV enzyme responsible for the transcription of HIV RNA into DNA. The licensed RTIs (zidovudine, didanosine, zalcitabine, lamivudine and stavudine) are of limited potency and durability of effect, with viral load suppressions of perhaps 0.5 to 0.8 logs for six months[13,14]. Combinations tend to produce CD4 and viral load responses of greater magnitude and duration[15]. The licensed NNRTIs are nevirapine, delavirdine and efavirenz.

Monotherapy with these rapidly becomes ineffective; they are more useful when used with two RTIs[16,17].

The third class, the protease inhibitors (or "PIs") prevent cleavage of the nascent HIV protein after genomic replication and translation have occurred. This results in the formation of immature virions, which lack the ability to infect other cells. Members of this family of drugs include saquinavir, ritonavir, indinavir and nelfinavir. These drugs are much more potent *in vivo*, with typical viral load suppressions of 1-2 $\log_{10}$, often lasting many months even when used alone. These are most effectively used in combination with nucleoside analogues or other protease inhibitors, providing potent suppression of HIV for extended periods of time[18].

1.3. Therapeutic Monitoring

There are difficulties with the evaluation of *clinical* effect of antiretroviral drugs:

1. Therapy is directed towards improvement in immune function and therefore reduction in *risk* for illness, not symptomatic control.

2. Antiretroviral therapy is provided at any point in the course of HIV infection, although the illnesses being prevented are not expected to occur for several years, as they are most typical of advanced HIV infection (with CD4 cell counts below 200 or especially 100 cells/μL[19], where normal counts exceed 600). This is similar to the situation with other diseases, in which a manifestation of the disease process is measured, with effects on health being delayed months or years. For example, the treatment of hypertension is measured by the reduction of systolic or diastolic blood pressure; clinical benefits, however, are not immediate. The reduction in cardiovascular morbidity and mortality produced by these therapies can only be determined after years of treatment.

As the course of HIV infection may be ten years or longer, the conduct of clinical trials of antiretroviral therapies would be extremely difficult if it were required that clinical endpoints (the development of HIV-related illnesses or death) were assessed. As such, monitoring of surrogate marker response are used. It follows, then, that the relationship between changes in these markers and reduction in likelihood of illness and death must be established.

Clinical guidelines suggest that surrogate marker responses should be assessed four, eight or twelve weeks after initiation of antiretroviral therapy[20,21]. Previous studies examining this relationship have mostly addressed the magnitude of change in the surrogate marker in response to therapy as a predictor of clinical response. However, these may be misleading, as the duration of response is not considered. It is well recognized that some antiretrovirals may strongly, but transiently, suppress viral load (ie, non-nucleoside reverse transcriptase inhibitors). Similarly, models based on the duration of response may be inaccurate, as the inferior potency of many antiretrovirals (including most of the nucleoside analogues) is not considered. Modelling techniques based on a combination of these factors may be superior. One option may be an analysis more typical of pharmacokinetic studies.

Pharmacokinetic studies typically examine the plasma concentration profile of a pharmaceutical compound, taking into account its absorption, distribution and elimination. The following indices are determined (Figure 1 and 2, page 14):

T_{max}: time to maximum change in concentration from baseline

C_{max}: magnitude of maximum change from baseline

AUC_t: area under the concentration curve to time t

T_{min}: time to return to baseline

Figure 1

Surrogate Marker (CD4) Response Considered as a Pharmacokinetic Curve

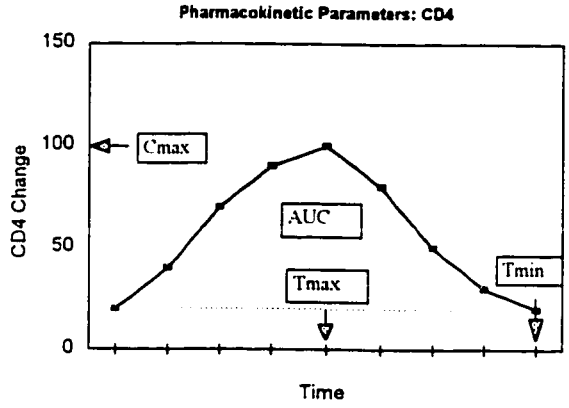
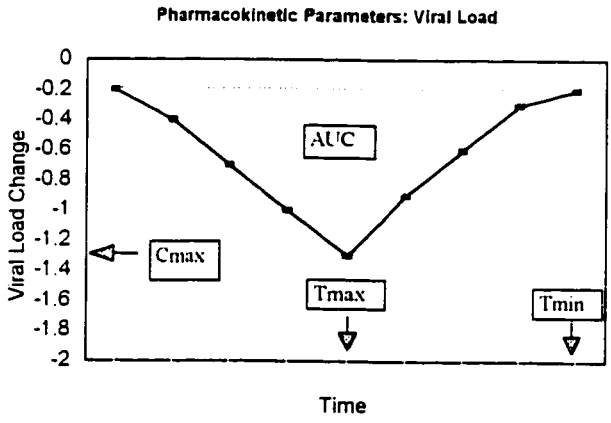


Figure 2

Surrogate Marker (Viral Load) Response Considered as a Pharmacokinetic Curve



C_{max} corresponds to the peak surrogate marker response. T_{max} may be difficult to interpret, as its relationship to drug activity is not immediately obvious. T_{min} represents the durability of response, but does not take into account the magnitude of response.

It may be that the AUC_t is the surrogate marker response that best takes into account the magnitude

and durability of response. However, there are no reports in the literature of the association between AUC and clinical outcome.

This association will be assessed in this thesis using the database from Abbott study M94-247. This randomized, double-blind, placebo-controlled study of the protease inhibitor ritonavir in advanced HIV infection was initiated on April 3, 1995. HIV-infected persons with a CD4 cell count below 100 cells/μL were assigned to receive ritonavir or a matching placebo in addition to their usual antiretroviral therapy. The primary endpoints in this study were survival and the occurrence of new or selected recurrent major opportunistic illnesses. These endpoints were scrutinized in a blinded manner by an independent committee and were required to fulfill pre-determined diagnostic criteria.

A total of 1090 subjects were enrolled in 67 centres in 10 countries. The study was stopped early when an interim analysis revealed that those assigned to the ritonavir arm experienced a survival benefit (hazard ratio for death = 0.669, p = 0.007) and fewer AIDS-defining illnesses or deaths (hazard ratio for death = 0.507, $p < 0.001$)[22].

Although all subjects had CD4 and CD8 monitored during the course of the study, monthly viral load measurements were only carried out on the first 159 with baseline HIV RNA > 15,000. The remaining study subjects had monthly visits for testing of CD4, CD8 and clinical assessment. After 16 weeks, those who experienced a new or selected recurrent opportunistic illness were provided with open label ritonavir; as well, other antiretrovirals could be changed beyond week 16 at the discretion of the treating physicians. After the study was closed, open label ritonavir was provided to all study subjects

1.4. Hypothesis

By taking into account magnitude and duration of surrogate marker response, the area under the curve of the response will be superior to the peak response, or response at any individual time point, in terms

of association with clinical outcome. The intent of this thesis, then, is to examine the association between time period, peak and AUC surrogate marker data from the M94-247 with clinical outcome.

The questions to be addressed are:

Question 1. How do the parameters AUC, C_{max}, T_{max} and T_{min} compare with each other, and with time point surrogate marker changes, in terms of association with clinical outcome?

Question 2. Which time point of which surrogate marker is most associated with clinical outcome?

Question 3. What is the diagnostic performance (predictive values and likelihood ratios) of the clinical and overall surrogate marker responses of greatest specificity?

Literature Review

The number of surrogate markers investigated for HIV infection is extensive. At present, the main surrogate markers measured in clinical trials are the CD4, CD4% and HIV viral load. In addition to these surrogate markers, there are a number of other factors implicated in the progression of HIV infection:

Patient:	patient age at the time of infection
	past opportunistic illness
Therapeutic:	concomitant medications
	compliance

This review will focus upon the literature about the major surrogate markers, in particular CD4, CD4%, CD8, CD8% and viral load. The role of β2-microglobin, serum neopterin, SI/NSI phenotype, patient age, past history of opportunistic illness, concomitant medications, and compliance may be touched upon, but are not the focus of this review. Only published literature will be cited; there are a total of 45 such publications that will be reviewed, which address surrogate markers and the natural history of HIV, and their role in monitoring therapy.

2.1 Introduction

Repeat measures of a single blood sample have been found to vary by 5-25%, the range being inversely proportional to the absolute count[23]. A second group[24] followed a cohort of 1020 untreated persons for two years, and found the coefficient of variation within individuals to be 25%, also inversely related to the absolute count; in 6% of subjects, a second count within 8 weeks was half or double the first. The rate of decline averaged 14.3% per year, independent of baseline, but actually rose in 29% of subjects.

Longitudinal data document the rate of decline of CD4 cells over time[25-28]. This tends to be slow and constant when asymptomatic, but increases when clinical illness supervenes. For instance, the Schellekens study[28] noted an increase in slope of decline when the CD4 cell count reached 400 cells/μL. As well, the likelihood of developing HIV-related illness is closely related to the CD4 cell count, with the risk for any of these illnesses, in general, increasing with decreasing CD4 cell count[19,29]. In one study[28] the mean CD4 at the time of first AIDS-related illness was 140 cells/μL (90% confidence interval 30, 310).

Several authors[30,31,32] have demonstrated that the viral load is inversely proportional to the CD4 cell count, with wide variability in viral load for any CD4 range. Hughes *et al*[30] found the viral load to increase by 0.39 $\log_{10}$ for every decrease in CD4 cell of 100. The standard deviation of the viral load (by PCR) was found to be 0.19 $\log_{10}$, independent of level of viremia; the variance of the bDNA assay was 0.4 $\log_{10}$[33]. Although the risk of opportunistic illness is not as closely tied to the viral load as to the absolute CD4 cell count, the risk does increase for increasing viral loads within any CD4 range. Several authors have reported increasing viral loads immediately prior to opportunistic infection[34] or bacterial pneumonia[35], with decreases after treatment, implying that these changes are secondary to the illness, not causative.

2.2 Surrogate Markers and Natural History: Studies Not Assessing Viral Load

The importance of baseline CD4 cell count as a prognostic factor for the development of AIDS or death has been demonstrated repeatedly.

In an early study, Fahey *et al*[36] looked at the prognostic importance of CD4 and CD8 cell counts, CD4:CD8 ratio, p24 antigen, and four markers of immune activation (neopterin, soluble IL-2 receptors, IgA and β2-microglobin) for the development of AIDS in the Los Angeles cohort of the Multicenter AIDS Cohort Study (MACS). This cohort of at-risk gay men was recruited in four

American cities in 1984-5; they were followed semi-annually with HIV tests (if HIV-negative at entry into the study), CD4 cell count and routine biochemical and hematologic studies. Plasma was also stored for future use, including viral load testing, which became available in the mid-1990's. Using the Kaplan Meier method and Cox Proportional Hazards Modeling, Fahey determined that the most parsimonious combination of variables for prediction of the progression to AIDS in this untreated group was the CD4 cell count and serum neopterin. As this study was conducted in the 1980's, when little was available for the treatment of HIV or prevention of opportunistic illness, it represents a virtual natural history study.

A follow-up study using the MACS cohort[37] assessed which factors, independent of the baseline CD4 cell count, would predict the development of AIDS within 24 months. Controlling for baseline and six month change in CD4 with a multivariate Cox model, it was found that a low baseline CD4%, decline of the CD4% in the first six months of observation, and the development of thrush or fatigue were independently associated with the development of AIDS.

Another early study of HIV-infected injection drug users, by Page *et al*[38], demonstrated, using the same statistical methodology in a multivariate model, the significance of the association of low CD4 and elevated IgG or IgA at baseline with death.

2.3 Surrogate Markers and Natural History: Studies Assessing Viral Load

Many of the studies assessing the contribution of CD4 and viral load in the natural history of HIV infection are sub-analyses of the same cohorts, and therefore examine the same patients. Nonetheless, the weight of evidence supports the superiority of the viral load as a prognosticator, especially in the earlier stages of HIV infection.

The earliest published study assessing the relationship between viral load and clinical illness or

survival is that of Mellors *et al*[39]. This group compared HIV viral load (by bDNA), p24 antigen, neopterin and β2-microglobin in terms of association with the development of AIDS or decline in CD4 (defined as a statistically significant negative slope over all CD4 measures), using logistic regression, in a cohort of 62 men in the Pittsburgh portion of the MACS. Univariate analysis demonstrated a strong association of AIDS or CD4 decline with higher viral loads or p24 antigenemia. Nineteen of 23 with a stable CD4 cell count had an undetectable viral load, whereas only 10/39 with progressive CD4 decline or AIDS had this level of viremia. If at least one viral load measure was positive within two years of seroconversion, the risk of AIDS before the end of the study was 45% for those with CD4 cell counts above 500 cells/μL, but 86% for those with CD4 cell counts below this threshold. In multivariate analysis with AIDS as the outcome, only the viral load was significantly associated.

Mellors further investigated this relationship using the entire Pittsburgh group of MACS[31], and the full cohort[40]. In the former, he compared the prognosis for time to AIDS and time to death for the quartiles of baseline CD4 and viral load by Kaplan Meier curves. The discrimination was better for the viral load quartiles: the proportion developing AIDS at 5 years was 8%, 26%, 49% and 62%, respectively, for the lowest to highest viral load quartiles. The CD4 quartiles were less discriminating; only the lowest CD4 range discriminated from any other quartile in time to AIDS or death. The entire cohort was assessed in a similar manner[40], with the baseline CD4 cell count divided into rough quartiles (cut points of 200, 350, 500 and 750 cells/μL), as were the viral loads (cut points 500, 3000, 10,000 and 30,000 copies/mL). Analysis was performed with Kaplan Meier curves and Cox modeling. This study revealed a monotonic relationship between viral load and rate of CD4 decline. Within each CD4 or CD4% category, the viral load provided further discrimination of time to AIDS and survival. Overall, the baseline viral load was more strongly associated with the development of AIDS than CD4 or any other surrogate marker. When the baseline viral load was assessed in a similar manner in a cohort of hemophiliacs (followed from 1979 to 1995), a similar discrimination of risk of AIDS was found[41]. In this last study, the age-adjusted relative hazard for AIDS was 14.3 (95% CI 1.9-105.6) for those with

baseline viral load greater than 10,000 copies/mL, as compared to less than 1000 copies/mL.

Craib *et al*[42] followed a cohort of 79 HIV+ gay men in Vancouver for a median of 11.5 years. The rate of progression to AIDS was 69% and 34%, and mortality 61% and 27%, for those with baseline viral loads above and below the median (3040 copies/mL), respectively. Vlahov[43] looked at a larger cohort of 522 HIV-infected persons. With an outcome of AIDS defining illness or infection-related death, univariate analysis revealed a significant association with both baseline CD4 and viral load. Otherwise, increasing viral load within any CD4 cell range was associated with increased progression.

Analysis of a smaller cohort of 73 Swiss patients[44] revealed that, in a multivariate analysis, mortality was better predicted by the baseline CD4 cell count (RH for each 100 cell/μL increase 3.5, p=0.003) than by viral load (p=0.28). This is in contrast with a small study by Pedersen *et al*[45], in which stored sera from 93 seroconverters (taken 6-24 months after infection) was assessed, with over ten years of follow-up. Multivariate analysis revealed the viral load to be most strongly associated with the progression to AIDS, and the CD4 cell count less so. Similarly, a small French study[46] of 36 asymptomatic HIV-infected persons found, in a multivariate analysis, baseline viral load was the best predictor of progression to illness.

A small subset of untreated HIV-infected persons will survive disease-free for a prolonged period, often maintaining normal or near normal CD4 cell counts for ten or more years ("non-progressors"). Spijkerman *et al*[32] studied 77 men who had been free of AIDS for at least 8 years. There was an inverse relationship between baseline CD4 and viral load, and a positive relationship between viral load and rate of CD4 decline. A few did progress to develop AIDS; multivariate analysis revealed an association with the baseline CD4 cell count only. This is in contrast to a small study by Pantaleo *et al*[47], which demonstrated 20-fold lower viral loads among non-progressors compared to controls, with strong cell-mediated immune responses against HIV. An uncontrolled study of 10 non-progressors

confirmed the very low viral loads among these individuals[48]. Although rare individuals may have a mutant, non-virulent strain of HIV, it is likely that most non-progressors have very low viral loads by virtue of brisk anti-HIV immune responses.

A subset of HIV-infected persons progresses quickly to illness and death. Farzadegan *et al*[49] looked at the CD4, CD8 and viral load of seventeen men from MACS who developed AIDS within three years of seroconversion (as compared to 42 who developed AIDS 6 or more years after seroconversion). The baseline viral load measures were one $\log_{10}$ higher in rapid progressors, and their CD4 cells declined more rapidly thereafter. The viral load rose ten fold thereafter in the rapid progressors as compared to slow progressors.

HIV infection in children is a much different disease, and there is little reported about the association between surrogate markers and disease development. Shearer *et al*[50] looked at the importance of the viral load, but not the CD4 cell count, in 106 HIV-infected infants. Those who became ill quickly after birth had the highest post-partum viral load peaks and viral loads thereafter; those with the highest peaks had the fastest progression to illness. Mofensen *et al*[51] assessed the relationship between CD4 and viral load with mortality risk for children infected by intravenous gamma globulin. In a multivariate analysis, both a lower baseline CD4% and higher viral load were independently associated with death. In a time-dependent model, a one $\log_{10}$ increase in viral load led to a relative risk of 2.8 (95% CI 2.1, 3.6), while a 5% absolute decrease in CD4% had a RR of 1.3 (95% CI 1.2-1.5).

2.4 Surrogate Markers and Response to Therapy

A number of published studies have assessed the relationship between surrogate markers and clinical outcome, mostly in response to single or double RTI therapy. None have assessed the association with protease inhibitor therapy. Although not a standard therapy now, AZT has been demonstrated to reduce the likelihood of clinical illness or death in persons with advanced HIV, whereas follow-up

therapy with ddI or ddC has not demonstrated a survival benefit. Studies of surrogate marker responses from these treatment approaches parallel the clinical findings: treatment related CD4 changes are associated with clinical response to first line AZT therapy, but not second line ddI or ddC.

The predictive value, but inadequacy as well, of the CD4 cell count was highlighted by Choi *et al*[52]. This group analyzed an early AZT study (ACTG 019) and found that each decrease in baseline CD4 of 50 cells/μL was associated with a RR of disease progression of 1.75 ($p<0.001$). However, AZT's effect on CD4 cell counts accounted for only 0-37% of the clinical benefit.

Jacobsen *et al*[53] used data from a randomized study of two doses of AZT to perform a nested case control study of the predictive power of CD4, CD8, p24 antigen, β2-microglobin, neopterin, and soluble IL-2, CD4 and CD8, for development of ARC, AIDS or death. Cases and controls were matched for duration of treatment and dose of AZT, length of follow-up, and baseline CD4 cell count. Viral load was not assessed. Univariate analysis revealed that baseline levels of all the surrogate markers except for soluble CD4 and CD8 were associated with progression; higher baseline CD8 was associated with a worse prognosis. Treatment-related changes in CD4, CD4% and β2-microglobin were associated with progression as well; the association was strongest for the CD4. Multivariate analysis revealed that only the treatment-related CD4 cell count and β2-microglobin changes were predictive of illness.

Graham *et al*[54] looked at the association between early (within 6 months) CD4 rises and AIDS or survival with AZT monotherapy. AZT led to a mean 17 cell/μL increase in CD4 over 6 months, as opposed to the 30 cells/μL drop in those not taking this drug. After correcting for multiple baseline variables, early CD4 increases were found to be significantly associated with reduced progression to AIDS (rel. hazard 0.71 for each 100 cell/μL increase, $p=0.0001$) and increased survival (RH of death 0.78 per 100 cell/μL increase, $p=0.004$). A follow-up study[55] on the same cohort used Cox

proportional hazards modeling to look at the value of CD4 cell count, neopterin and β2-microglobin in AZT monotherapy. At baseline, CD4 cell count and freedom from major or minor opportunistic illness were associated with survival. Changes in CD4 cell count and continued lack of symptoms of HIV infection were significantly associated with reduced development of AIDS and increased survival.

The relationship between CD4 and disease or death was examined for ddI and ddC by Goldman *et al*[56], using data from a study of these drugs after failure of AZT. These authors calculated the slope between the baseline and CD4 at month 2; responders were defined as having a positive slope. Only slight initial increases in the CD4 cell count were seen among those assigned ddI; it fell in those assigned ddC. The CD4 counts declined after two months. There was a slight excess of deaths in the ddI group. A probit analysis, using treatment group, previous AIDS diagnosis and baseline Karnofsky score as covariates, demonstrated that only the baseline CD4 cell count was associated with death.

Mathematical modeling of the relationship between CD4 and viral load in response to indinavir therapy[57] found that the return to baseline of the CD4 cell count was related to the baseline CD4 count (r^2=0.86, $p<0.001$), and less so the decline in viral load (r^2=0.60, $p<0.01$). Simultaneous modeling revealed that the majority of variability in CD4 return to baseline was attributable to the baseline CD4 level, and that any effect of the viral load decline was at 50% of maximal with only 0.2 $\log_{10}$ decline. This suggests that the CD4 cell count is potentially misleading, as its duration of change is so influenced by minimal antiviral effect.

Both CD4 and viral load responses to therapy have been assessed in a number of studies. In general, CD4 and viral load responses are inversely related, but the range of CD4 responses for any viral load change is very wide. For instance, Marschner *et al*[58] demonstrated that, among 317 subjects from various studies, a viral load decline of 0.5 $\log_{10}$ was associated with a range of CD4 responses, from a decrease of 271 cells/μL to an increase of 261 cells/μL.

The independent but additive effect of CD4 and viral baseline levels and treatment induced changes was documented in two studies by O'Brien *et al*, and the importance of durability of response was highlighted. The first study[59] examined the relationship with AZT therapy, using data from the Veterans' Affairs 298 study. All subjects who had at least one baseline and one follow-up measure of both surrogate markers were studied, using Kaplan Meier curves, Cox Proportional Hazards Modelling and life tables. The markers were examined in terms of mean change from baseline in the six months thereafter. Multivariate analysis revealed that only baseline CD4 and viral load were associated with progression or death: a mean viral load increase of 0.5 $\log_{10}$ was associated with a relative risk of 1.27 for death, whereas a CD4 increase of 35 was associated with a relative risk of 0.85 A six month average increase in viral load of 0.5 $\log_{10}$ was associated with a RR of death of 1.5, whereas a CD4 increase of 35 cells/μL was associated with a RR of 0.83. A 75% decrease in viral load was associated with a RR of 0.44 for progression to AIDS (p=0.009) in multivariate analysis, and a CD4 increase of 10% resulted in a RR of 0.48 (p=0.007). More of the treatment effect was accounted for by viral load changes than CD4 changes; 79% of the treatment effect was accounted for by the two together.

Further work in the same cohort[60] demonstrated the additive nature of the surrogate marker responses, and the importance of duration of response. These authors looked at CD4 or viral load changes averaged over the first six months of therapy or placebo with the endpoint of AIDS. Multivariate analysis demonstrated the RR of AIDS was 0.70 for each mean 6 month averaged decrease in viral load of 0.5 $\log_{10}$ copies/mL; the RR for a CD4 increase of 35 cells/μL was 0.82 ($p<0.001$ for both). There was no interaction; the RR of AIDS was 0.33 with both surrogate marker changes together, and 2.3 if neither occurred as compared to at least one change occurring. The RR of AIDS was 4.28 if the viral load return to baseline within 6 months, as compared to after this point; no such relationship was found for the CD4 cell count.

These studies brought to light the greater importance of viral load in monitoring therapy, which was confirmed by later publications. Coombs *et al*[61] examined viral load data from ACTG 116B/117, in which a survival benefit was found when switching from prolonged AZT to ddI. The baseline viral load was found to be an important prognosticator for the development of AIDS, even when adjustments were made for multiple other variables (RH 1.26 for each doubling in viral load, p=0.02). A treatment-related 50% reduction in viral load was associated with a RH for illness of 0.73 (p=0.07). A similar analysis[62] of ACTG 116A (ddI for AZT-naive persons) demonstrated the independent prognostic value of baseline CD4 and viral load, as well as the poor prognosis associated with on-treatment viral load increases (RH 1.45, 95% CI 1.02, 2.05). Hughes *et al*[30] assessed the predictive value of viral load and CD4 changes in ACTG 241, which compared double RTI therapy with double RTI-single NNRTI therapy. In terms of treatment related changes, only week 8 viral load change was associated with progression to AIDS; a one log decline was associated with a 52% reduction in progression.

Several studies have assessed the relationship between surrogate markers and clinical illness in ACTG 175, a study of combined vs single RTI therapy. Katzenstein *et al*[63] used multivariate Cox proportional hazards modeling to determine that higher baseline viral load, lesser degrees of treatment-related viral suppression, and SI phenotype were associated with progression to AIDS or death. After adjustment for these, the CD4 cell count did not have a statistically significant association with the endpoint. Fiscus *et al*[64] also examined the relationship between CD4, viral load and disease progression (new opportunistic illness or death) using KM and Cox methods. Only baseline and week 8 changes in viral load were associated with opportunistic illness or death in multivariate analysis. The presence of the SI variant at baseline, or its development, was also associated with increased disease progression. Greater reductions in viral load were associated with higher CD4 responses, and "undetectable" viral loads at any point were associated with reduced progression. This was the first publication to assess the effect of "undetectable" viral loads (meaning the viral load was too low to be accurately quantitated

by the assay), which has now become the gold standard surrogate marker response. A variant of this study was published by Lathey *et al*[65]. This study looked at viral load, CD4 and SI phenotype, but also the infectious (culturable) HIV titre and p24 antigen. Multivariate analysis found that disease progression to AIDS, death or CD4 decrease of 50% was associated only with viral load and infectious HIV titre, not CD4 cell count.

Both treatment-related CD4 cell count and viral load were associated with the development of AIDS in a multivariate analysis of two clinical trials of the reverse transcriptase inhibitor lamivudine[66]. This was confirmed by an analysis of the CAESAR study[67], in which 1840 patients were randomized to placebo, lamivudine or lamivudine plus loviride. Treatment-related changes in CD4 and viral load were both associated with progression to AIDS: a viral load reduction of 1.0 $\log_{10}$ between 12 and 20 weeks of therapy was associated with a RR of 0.51 (95% CI 0.30, 0.87); a 50 cell/μL rise in CD4 was associated with a RR of 0.43 (95% CI 0.26, 0.71).

Finally, Marschner *et al*[58] looked at the surrogate marker responses of 1330 subjects assigned to a variety of antiretroviral medications in seven ACTG trials, including ACTG 175 (see above). The time period of observation was set at 24 weeks, and the responses at weeks 8 and 24 were examined by Kaplan Meier curves and Cox Proportional Hazards Modelling for the endpoint of AIDS-defining illness. There was a strong independent predictive value for both baseline CD4 and viral load: a one log reduction in viral load was associated with a 53% reduction in progression, and a CD4 increase of 100 cells/μL was associated with a 60% decrease in progression. After adjustment for baseline viral load, a one log reduction at 24 weeks reduced progression 72%, with a gradient of effect with increasing degrees of viral load suppression. This gradient of effect was also present within individual CD4 cell ranges. A lack of response of CD4 or viral load at week 24 was associated with an 86% increase in risk of progression compared to any change. Multivariate analysis demonstrated that each treatment-related reduction in viral load by one $\log_{10}$ was associated with a 51% reduction in

progression, and each CD4 increase of 100 cells/μL was associated with a 24% reduction in progression.

2.5. Summary

In summary, both the CD4 and viral load are of significant predictive value for the prognosis of untreated HIV infection. In multivariate analysis, no other surrogate marker is consistently associated with clinical outcome. Very low CD4 cell counts are associated with a significant risk of AIDS or death, whereas the viral load is more broadly discriminatory, as increasing quartiles are associated with increasing risks of progression in general, and within any CD4 range. In treatment, there is good evidence that changes in both the CD4 and viral load have independent predictive value, although the viral load may be more consistent across studies and of greater association in each. As well, there is evidence that durability of response is associated with clinical outcome.

Methods

3.1. Overview

This study was intended to assess the relationship between clinical outcome and (1) clinically useful surrogate marker measures (at specified time points after treatment initiation), and (2) peak and area under the curve of surrogate marker response, primarily for subjects assigned ritonavir.

Logistic regression was used to determine the specificities of these surrogate marker changes, which were then compared. For the time period and overall surrogate marker responses of greatest specificity, the odds ratio and positive and negative predictive values and likelihood ratios were calculated. The time frame of this study is 40 weeks, with censoring of any surrogate marker or clinical outcome data occurring beyond this time. All analyses were performed on a Hewlett Packard Pentium 120 computer, using the SPSS 7.5 for Windows statistical package.

3.2. Study Population

Subjects for M94-247 were enrolled in 67 centres in ten countries in North American, Europe and Australia. A total of 1716 subjects were screened and 1090 eventually randomized. Six hundred and twenty-six potential subjects were excluded for the following reasons:

371 with abnormal lab results

116 with CD4 cell count > 100 cells/μL

45 withdrew consent

26 with acute illness/ clinical deterioration precluding randomization

2 died

15 requiring protocol-prohibited drug

34 other reason

17 reason not specified

All subjects were used for this analysis if baseline surrogate marker data were available. The characteristics of the subjects used in this analysis are presented in the "results" section.

3.3. Data Description and Transformation

Outcome Variable

The outcome variable for this analysis could be one of three nominal variables:

I. First (or selected recurrent) opportunistic illness

II. Death

III. First opportunistic illness or death

The strengths and weaknesses of these are outlined in Table 1.

Table 1

Strengths and Weaknesses of Possible Outcome Variables

Outcome	Strengths	Weaknesses
AIDS-defining illness	- strongly related to immune status and therefore drug effectiveness - carefully scrutinized - clinically significant	- moderately large number of subjects
Death	- well-defined - clinically significant - deaths may be related to HIV or study drug but not OIs (HIV cardiomyopathy)	- small numbers - some deaths may be unrelated to HIV (suicide, hepatitis) or immune status
AIDS-defining illness or death	- as for "deaths" - considers more possible serious events - largest number of subjects	- potential inequality of endpoints (some OIs are of limited morbidity) - some deaths may be unrelated to HIV (suicide, hepatitis) or immune status

The endpoint of first (or selected recurrent) opportunistic illness was chosen (see discussion). For the entire cohort, 258/1086 (23.8%) subjects experienced an opportunistic illness in the period of follow-

up. In the group assigned to receive ritonavir, 88/541 (16.3%) developed an opportunistic illness.

Covariates

The baseline variables of concern are those with a scientifically plausible association with the endpoint of opportunistic illness. Those considered for this study are the following

I. Past major opportunistic illness (nominal)

II. Past minor opportunistic illness (nominal)

III. Present reverse transcriptase inhibitor (RTI) use (nominal)

IV. Present number of reverse transcriptase inhibitors used (ordinal: 0, 1, 2, 3)

V. Present number of reverse transcriptase inhibitors used (ordinal: 0, 1, ≥2)

VI. Reverse transcriptase inhibitor(s) added to week 16 (nominal)

VII. Reverse transcriptase inhibitor(s) added to week 40 (nominal)

VIII. Subject age (continuous)

Information on the other baseline variables of SI/NSI phenotype[68,69,70] (syncytium inducing/non-syncytium inducing) and subject compliance with medication[71], which are commonly associated with clinical outcome, were not available from the sponsor of the study.

The surrogate markers to be studied, all continuous variables, are:

I. CD4 T lymphocyte cell count

II. CD4 %

III. CD8 T lymphocyte cell count

IV. CD8%

V. HIV RNA level ($\log_{10}$ transformed)

Surrogate marker responses were analysed as the following variables:

I. Baseline

II. Change from baseline to week 2

III. Change from baseline to week 4

IV. Change from baseline to week 8

V. Change from baseline to week 12

VI. Change from baseline to week 16

VII. Peak response (or nadir response, in the case of viral load) to week 16

VIII. Time-averaged area under the curve to week 16 or endpoint before week 16 ($TAUC_{16}$)

IX. Time-averaged area under the curve to week 40 or endpoint before week 40 ($TAUC_{40}$)

The baseline, peak and time-averaged AUCs were assessed individually for the subjects assigned ritonavir and placebo, and all subjects considered together. The week 2 to week 16 responses were assessed for the ritonavir-assigned subjects only; there is little meaning to time period surrogate marker changes for individuals randomized to placebo, as these would presumably be random changes in the surrogate marker. The CD4 and CD8 T lymphocyte cell counts (herein refer to as CD4 and CD8 cell counts, respectively), and the HIV RNA level (herein referred to as viral load), are measured directly. The CD4% and CD8% are calculated as the proportion of lymphocytes represented by these cell lines, respectively.

[The thesis proposal had discussed analysis of the association between time to peak or time to nadir and clinical outcome. However, upon examination of the database, it is clear that the large variability in surrogate markers would preclude the consistent determination of these points. For instance, it is not unusual to see a slight decline in CD4 early in the study, regardless of therapy received, thereafter followed by a rise, usually in response to therapy. For this reason, these variables were not examined in this thesis].

Distributions of Baseline Variables and Covariates

As needed, distributions of covariates was determined by examination of a histogram of the frequency of covariate value. An appearance of normality was confirmed by the Kolmogorov-Smirnov test.

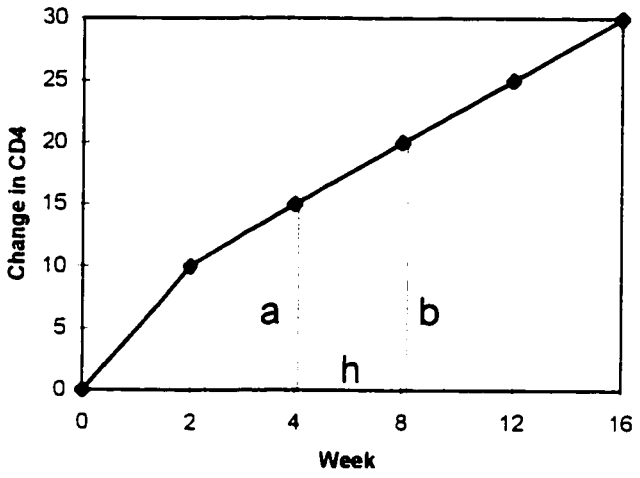
Time-Averaged Area Under the Curve

The area under the curve for the surrogate markers in this study was calculated with the trapezoidal rule:

$$AUC = \sum \frac{(a + b) \times h}{2}$$

Figure 3

The Trapezoidal Rule



where a = change from baseline at start of interval

b = change from baseline at end of interval

h = length of interval

The time-averaged area under the curve was calculated as follows:

$$\text{Time-averaged AUC} = \frac{AUC}{x}$$

where x = weeks of follow-up

TAUCs were determined for each variable to week 16 (herein referred to as $TAUC_{16}$): the study protocol required that there be no change in antiretroviral therapy to this point. These TAUCs, therefore, should represent the effect of the study drug only, as opposed to the study drug plus whatever other therapies were started at the discretion of the investigators.

The TAUC was also determined for the entire 40 week period under study (herein referred to as $TAUC_{40}$): this will allow for analysis of the surrogate marker response to the therapeutic approach, which may involve addition or discontinuation of ritonavir or other antiretrovirals. This will offer little in terms of determining ritonavir-related changes; it will assess the clinical response to surrogate marker changes in general.

There are several potential difficulties with AUC calculation:

1. Different periods of follow-up. Study visits were scheduled at weeks 0, 2 and 4, and then every four weeks. Relatively few subjects continued to be followed to 40 weeks because of death, development of illness, or loss to follow-up. To allow for appropriate correction for this, the time-averaged AUC was used.

2. Irregular periods of follow-up. This study will compare the TAUCs of those who do not develop an endpoint as compared to those who do; the data will be censored at the point of illness for the latter. However, this point of illness may not be at a study visit; for instance, it may be at day 32, which is four days after the week 4 visit. The options available would be two-fold:

A Exact analysis: calculation of the projected TAUC to the point of censoring based on that of the previous interval

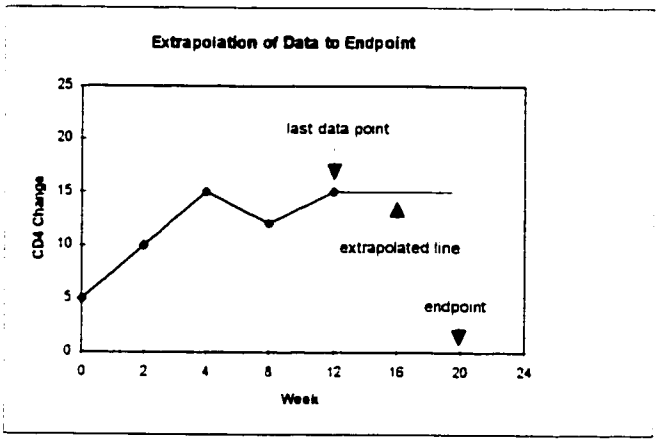
B Truncated analysis: calculation of the TAUC to the study visit immediately preceding the endpoint

Both of these were performed for the CD4 cell count for all subjects, and the corresponding Pearson correlation coefficient was found to be 0.902 ($p < 0.001$). Because of this close association, and because of the difficulty of calculation required for the exact analysis, the truncated analysis was chosen.

3. Missing data. Subjects with missing baseline values were excluded from the analysis, as no change from baseline could be calculated. Otherwise, missing data points were extrapolated as being on a straight line between adjacent available data points. For calculation of the TAUC to the time of an endpoint (or, in this analysis, to the study visit before endpoint development) when data is missing immediately prior to the pre-endpoint visit, the last available surrogate marker measure was carried forward to the time pre-endpoint visit (Figure 4).

Figure 4

Method of Extrapolating Data to the Point of Clinical Outcome in the Case of Missing Data



3.4. Statistical Methods

Logistic Regression

Logistic regression was used to determination the association between surrogate marker changes and opportunistic illness, allowing us to answer the following question:

> "How is the change in x units of the surrogate marker in response to the initiation of therapy with ritonavir associated with reduction of y units in the odds of developing an HIV-related illness or death over the period of t weeks?"

Univariate logistic regression was used to determine whether there was an association between baseline variables and the outcome. A level of significance of 5% was used for this selection of baseline variables.

$$g(x) = \beta_0 + \beta_1 x_1$$

where x_1 = baseline variable and g(x) is the logit function

Next, logistic regression (univariate and with inclusion of significant baseline variables)was performed for each covariate. Again, the level of significance accepted for the model χ^2 was 0.05.

$$g(x) = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3$$

where x_1, x_2 = baseline variables

x_3 = surrogate marker response

Multivariate analysis was performed in two steps: the first entering the baseline variables of statistical significance, and the second using forward stepwise regression of the five surrogate markers at a given week (or peak, $TAUC_{16}$ or $TAUC_{40}$). The *p to enter* was 0.15, and *p to remove* 0.20.

Determination of Surrogate Marker Predictive Value

The observed vs expected classification table (adjusted for the number of the outcomes) provides a

direct output of the proportion of cases categorized properly with the logistic regression equation used. This yields the percent correct of those with outcome "0" (lack of opportunistic illness in this study), and percent correct with outcome "1" (development of opportunistic illness). These are the specificity and sensitivity of the prediction of outcome based on a logistic regression model using surrogate marker response.

The use of specificity in this study is clinically relevant. The use of a test with increased specificity would allow for exclusion of individuals without disease (or unlikely to develop a clinical endpoint in this study); those left would be likely to develop illness or be false-positives (as false positive rate = 1 - specificity), who could be monitored more closely or prophylactic measures taken. Specificity is defined as:

$$\text{specificity} = \frac{d}{b+d}$$

as defined from a standard 2x2 contingency table:

	Disease +	**Disease -**
Test +	a	b
Test -	c	d

Specificity is a binomial entity; as such, the 95% confidence interval is calculated as follows:

$$95\%\ CI = p \pm \left(z_{\alpha/2} \cdot \left(\frac{p(1-p)}{n} \right)^{1/2} \right)$$

where p = specificity

n = sample size (b + d from the contingency table)

For the purposes of this thesis, we will use the specificity as the measure of surrogate marker response. In other words, we will determine which surrogate marker, or set of markers, is most associated with freedom from clinical illness. This will be determined, by examination of the specificity and 95%

confidence interval, for the clinically applicable surrogate marker response and for all possible surrogate marker responses.

Interaction

There is evidence from clinical trials that the virologic response to antiretroviral therapy may, in part, be dependent upon the baseline CD4 cell count or viral load. As well, it is generally felt that the CD4 response to antiretroviral therapy may be hindered by a low baseline CD4 cell count; there is little published evidence to support this. Therefore, the interaction between baseline and time period/peak/AUC changes in the same surrogate marker was explored for CD4, CD4% and viral load. As well, the viral load tends to increase as the CD4 or CD4% decreases[30,31,32]; this interaction was explored as well.

Interpretation of Results

Clinical practice requires "real time" assessment of surrogate marker responses. As such, the peak response (which can only be determined retrospectively, once all the data points are available) cannot be used in clinical management. As well, the TAUCs are determined retrospectively, and by their very nature, would not be amenable to use in clinical practice. As well, both Canadian and American guidelines suggest reassessment of the patient within 8 weeks of initiation of therapy, and the standard of clinical care is to see patient every twelve weeks:

Canadian[20]: - plasma viral load should be measured 4-8 weeks after initiation or change of therapy
- plasma viral load and CD4 should be measured every 3 months thereafter

American[21]: - plasma and CD4 cell count should be performed at baseline, after 4 weeks, and every 3-4 months thereafter.

As such, the Week 16 response will be excluded from consideration as the best surrogate marker response for clinical use. Therefore, the surrogate marker responses assessed for clinical use were the Baseline and Week 2 to Week 12 responses. The surrogate markers of greatest clinical and overall specificity in those assigned ritonavir were assessed for the following:

1. Odds Ratio

The odds ratio for the development of opportunistic illness were calculated with the formula:

$$\Psi = e^{\beta 1 \cdot \Delta}$$

where Ψ = odds ratio

β_1 = estimated coefficient of the surrogate marker in the logistic equation

Δ = change in surrogate marker

2. Positive and Negative Predictive Value

The positive predictive value was calculated with the following formula:

$$PPV = \frac{a}{a + b}$$

where PPV = positive predictive value

a and b are taken from the 2 x 2 contingency table (see page 37)

The negative predictive value was calculated with the following formula:

$$NPV = \frac{d}{c + d}$$

where NPV = negative predictive value

c and d are taken from the 2 x 2 contingency table (see page 37)

3. Positive and Negative Likelihood Ratios

The positive likelihood ratio (+LLR), and negative likelihood ratio (-LLR) were calculated with the

following formulae:

$$+LLR = \frac{a}{a+c} \div \frac{b}{b+d}$$

$$-LLR = \frac{c}{a+c} \div \frac{d}{b+d}$$

where a, b, c and d are taken from the 2 x 2 contingency table (see page 38).

4. Receiver Operating Characteristic Curves

Receiver operating characteristic curves (ROCs) were developed for the clinical and overall surrogate marker responses of greatest specificity using source data, coded such that those subjects without data at the time point of interest were excluded from the calculations.

Statistical Significance

Unless otherwise specified, statistical significance is assessed at the 5% level.

4.1. Study Population

Of the available subjects from M94-247, 1086 subjects were used. Four were excluded because of lack of baseline surrogate marker data. The characteristics of the subjects are illustrated in Table 3 (page 43). The opportunistic illnesses encountered in this study are in Table 4 (page 44).

4..2. Baseline Variables

Potentially confounding variables were analysed separately for all subjects and those assigned to ritonavir, with the outcome of opportunistic illness (Table 2). Only the baseline variable "past major opportunistic illness" was found to have a statistically significant association with the outcome , but "present RTI use" was of borderline statistical significance for all subjects as well as those assigned to ritonavir. Therefore, these two variables were included as baseline variables in further analyses.

Table 2

Association of Baseline Variables with Opportunistic Illness in All Subjects and Those Assigned Ritonavir

	All Subjects				Ritonavir Subjects			
	LLRχ^2	Sig	β	OR	LLRχ^2	Sig	β	OR
Past Major OI	5.552	0.185	0.388	1.474	6.423	**0.011**	0.728	2.071
Past Minor OI	0.010	0.922			0.506	0.477		
Present RTI Use	2.897	**0.080**	-0.060	0.946	2.549	0.110	-0.429	0.651
Present # RTIs	0.953	0.812			2.478	0.479		
Present # RTIs (1)	0.221	0.896			2.473	0.290		
Pt. Age (cont.)	0.044	0.834			2.822	0.253		
Tx added (16 wks)	0.052	0.819			0.161	0.689		
Tx added (40 wks)	1.097	0.295			0.505	0.477		

LLRχ2 = log likelihood chi square
sig = level of statistical significance
β = coefficient
OR = odds ratio

Table 3

Characteristics of Subjects Used for Surrogate Marker Analysis

	Ritonavir	Placebo	All Subjects
Median Baseline CD4 (IQR) (cells/μL)	18 (10, 43)	22 (10, 47)	20 (10, 44)
Median Baseline CD4% (IQR) (%)	3.0 (1.5, 5.0)	3.0 (1.5, 5.5)	3.0 (1.5, 5.5)
Median Baseline CD8 (IQR) (cells/μL)	411 (245, 635)	408 (242, 677)	409 (243, 657)
Median Baseline CD8% (IQR) (%)	59.0 (47, 68.5)	60.5 (49.5, 69.0)	60.0 (48.0, 69.0)
Median Baseline Viral Load (IQR) (log_{10} copies/mL)	5.40 (4.98, 5.61)	5.23 (4.86, 5.53)	5.28 (4.92, 5.57)
Median Age (IQR) (yrs)	38 (34, 44)	38 (33, 44)	38 (34,44)
Past Major OI (%)	67.1	61.3	64
Past Minor OI (%)	88.4	91.7	90
Present RTI Use (%)	78.2	77.4	77.8
% male	92.1	91.4	91.5
% developing OI	16.3*	31.2*	23.8
% dying	4.6*	6.4*	5.5

* $p \leq 0.05$

Table 4

Occurrence of Opportunistic Illnesses in M94-247 by Type

	Number	% of Outcomes	% of Study Population
Candidiasis	60	22.8	5.5
Cervical Cancer	0	0	0
CMV Retinitis	37	14.1	5.1
Other CMV Infections	17	6.5	1.6
Coccidioidomycosis	0	0	0
Cryptococcosis	7	2.7	0.6
Cryptosporidiosis	6	2.3	0.6
Herpes Simplex	3	1.2	0.3
Histoplasmosis	2	0.8	0.2
Isosporiasis	0	0	0
MAC	22	8.4	2
Pneumocystosis	33	12.5	3
PML	6	2.3	0.6
Bacterial Pneumonia	2	0.8	0.2
Salmonellosis	0	0	0
Toxoplasmosis	4	1.7	0.4
Tuberculosis	0	0	0
AIDS Wasting Syndrome	11	4.2	1
HIV Encephalopathy	7	2.7	0.6
Kaposi's Sarcoma	27	10.3	2.5
Lymphoma	12	4.6	1.1
Other	7	2.7	0.6
Total	263	100	

CMV = cytomegalovirus

MAC = *Mycobacterium avium* complex

PML = progressive multifocal leukoencephalopathy

4.3. Description of Covariates

The descriptive statistics for the covariates is included in Appendix 2.

4.4. Correlation Between Covariates

The correlation between the covariates CD4 and CD4%, and between CD8 and CD8%, were analysed by means of Spearman rank correlation (Table 5).

Table 5

The Correlation Between CD4 and CD4%, and CD8 and CD8%, for All Subjects and Subjects Assigned Ritonavir

		Range of Significance of Correlation*
All Subjects	CD4 and CD4%	0.699-0.847
	CD8 and CD8%	0.270-0.683
Ritonavir Subjects	CD4 and CD4%	0.723-0.839
	CD8 and CD8%	0.291-0.665

* level of significance by Spearman Correlation, at different times of observation

The correlations, in general, are slightly greater for those assigned to ritonavir than for the study population as a whole, and greater for the CD4 and CD4% than for CD8 and CD8%.

4.5. Univariate Analysis

Univariate logistic regression, both unadjusted and adjusted for the significant baseline variables, was performed for the time period, peak and TUAC responses for all five surrogate markers, for all subjects, and for those assigned to ritonavir and placebo. The results are presented in Appendices 3-7.

4.6. Comparisons of Specificities

The specificities derived from the logistic regression equations of the surrogate markers adjusted for baseline variables are presented in Table 6a and 6b (page 44).

Table 6a

Specificities of the Surrogate Marker Responses Adjusted for Baseline Variables for Subjects Assigned Ritonavir

	CD4	CD4%	CD8	CD8%	VL
Baseline	46.00	47.86	35.98	31.49	58.21
Wk2	39.66	32.75	36.23	42.32	61.40
Wk4	50.42	49.71	39.42	32.95	45.45
Wk8	55.90	50.00	45.39	33.43	46.43
Wk12	55.02	54.97	50.31	38.82	26.00
Wk16	61.23	63.61	61.90	64.24	23.08
Peak	51.88	42.97	48.70	32.03	40.68
$TAUC_{16}$	56.68	69.63	62.11	54.66	77.97
$TAUC_{40}$	58.54	57.05	59.28	32.12	61.02

* shaded cells are of surrogate marker responses whose LLRχ^2 did not reach statistical significance ($p \geq 0.05$).

Table 6b

Specificities of the Surrogate Marker Responses Adjusted for Baseline Variables for All Subjects and Subjects Assigned Placebo

	Placebo					All				
	CD4	CD4%	CD8	CD8%	VL	CD4	CD4%	CD8	CD8%	VL
Baseline	43.34	47.12	47.47	34.94	46.67	45.11	47.39	44.65	33.00	29.46
Peak	46.89	36.33	42.44	44.69	51.22	45.77	48.20	49.64	33.38	32.00
$TAUC_{16}$	56.43	49.68	49.84	56.27	60.00	51.26	53.96	50.93	58.82	30.30
$TAUC_{40}$	52.50	50.32	51.77	56.91	60.00	50.00	55.78	58.37	39.02	45.45

* shaded cells are of surrogate marker responses whose LLRχ^2 did not reach statistical significance ($p \geq 0.05$).

Some general observations about these specificities may be made:

Ritonavir-Assigned Subjects

1. None of the viral load surrogate marker responses, except for the baseline, can be interpreted because of lack of statistical significance of the model χ^2. Most of the CD8% responses, as well, do not reach statistical significance.

2. There is a clear trend towards increasing specificity from baseline to week 16 for the remaining surrogate markers.

3. Considering time points up to 12 weeks (during which time initial surrogate marker testing would be performed after starting antiretroviral therapy), CD4 and CD4% are clearly superior to the included CD8 responses. These differences are less evident at 16 weeks.

4. Peak responses for any surrogate marker are inferior to the $TAUC_{16}$ and $TAUC_{40}$, as well as the 12 and 16 week responses for all the included surrogate markers.

5. The specificities of the $TAUC_{16}$ and $TAUC_{40}$ is as good as or better than any of the time period responses in almost all cases.

6. The $TAUC_{16}$ for the CD4% appears to have the greatest specificity (69.63%).

Placebo Subjects

1. Only the baseline CD4 cell count was associated to a statistically significant degree with the outcome of opportunistic illness. The degree of association between the other covariates and the outcome did not exceed that expected by chance alone.

All Subjects

1. None of the viral load results is of statistical significance.

2.. The baseline surrogate marker levels and peak response for any surrogate marker is inferior in specificity to the $TAUC_{16}$ and $TAUC_{40}$.

3. The difference in specificity between all subjects and those assigned ritonavir is variable in magnitude and direction.

4.7. Multivariate Analysis

Is a combination of variables superior to univariate analysis? This question was investigated for the ritonavir-assigned subjects as well as all subjects considered together. After entry of the baseline variables, the five surrogate markers at each time point or for each PK parameter were entered into a logistic regression equation using the forward stepwise method (see Methods). The results are illustrated in Appendix 8.

The surrogate markers were entered in several ways:

Entry 1.All five surrogate markers (CD4, CD4%, CD8, CD8%, viral load)

Entry 2:CD4, CD8, viral load

Entry 3.CD4, CD4%, CD8, CD8%

Entry 4.CD4, CD4%, CD8, CD8% (for those subjects with available viral load data)

By examination, several general statements can be made about the results:

1. Any regressions that include viral load as a possible covariate have very small sample sizes.

2 In only a few regression equations are multiple covariates included. Most include only a single covariate or none at all.

3. For most sets of equations, the univariate $TAUC_{16}$ and $TAUC_{40}$ have as high, or higher, specificity

than those of the time period surrogate marker responses.

4. In general, the specificities of ritonavir-assigned subjects is superior to those of all subjects considered together.

5. None of the specificities is superior to those of the univariate equations.

4.8. Interactions

For ritonavir-assigned subjects, several interactions were assessed:

1. Baseline surrogate marker and time point response, peak, $TAUC_{10}$ and $TAUC_{40}$ for the *same* marker (Table 7):

Table 7

Level of Significance of Interactions Between Baseline Surrogate Marker Level and Week 2 to Week 12, Peak or AUC Responses of the Same Surrogate Marker

	CD4	CD4%	CD8	CD8%	Viral load
Week 2	0.725	0.981	0.592	0.445	**0.038**
Week 4	0.860	0.703	0.519	0.703	0.350
Week 8	0.475	0.679	0.775	0.877	0.063
Week 12	0.212	0.921	0.051	0.494	0.899
Peak	0.965	0.641	0.186	0.972	**0.043**
$TAUC_{10}$	0.216	0.420	**0.018**	**0.008**	0.182
$TAUC_{40}$	0.130	0.212	0.893	0.445	0.152

(baseline surrogate markers on horizontal axis; in bold are interaction terms with p value ≤ 0.05)

2. Baseline CD4, CD4%, CD8 and CD8% with baseline viral load (Table 8).

Table 8

Interactions Between Baseline Viral Load and Baseline CD4, CD4%, CD8 and CD8%

	CD4	CD4%	CD8	CD8%
Viral Load	0.135	0.160	0.350	0.071

In summary, there is variable interaction between surrogate marker response and baseline surrogate marker level for persons assigned ritonavir. This does not effect the CD4 cell count and CD4%, and only the earliest viral load response. There is no significant interaction between baseline viral load and any other baseline measure, although there is borderline statistical significance of the interaction between baseline viral load and baseline CD8%.

4.9. Question 1

How do the TAUC and peak changes compare with each other, and with time point surrogate marker changes, in terms of association with clinical outcome?

Ritonavir-Assigned Subjects

All surrogate marker responses with their 95% confidence intervals are illustrated in Table 9 (page 50). All time point, peak and TAUC parameters were considered to determine which surrogate marker change, for research purposes, has the greatest specificity. The highest specificity is that of the $TAUC_{16}$ for the CD4%: specificity = 69.63% (95% CI 65.38, 73.88). There is overlap of the 95% CI with those of the week 16 CD4, CD4%, CD8 and CD8% responses, and with the baseline viral load. Based on these results, for persons assigned ritonavir, the 16 week TAUC of the CD4% is most strongly associated clinical response: the responses of the CD4, CD4% and CD8 increase over time and by week 16 approach the same specificity.

Placebo-Assigned Subjects

The only surrogate marker response associated, to a significant degree, with clinical outcome is the baseline CD4 (specificity = 43.34%; Table 10, page 51). This specificity is inferior to most of those for subjects assigned ritonavir.

All Subjects

For all subjects (Table 11, page 51), the AUCs are clearly superior, with the $TAUC_{16}$ for CD8% and $TAUC_{40}$ for CD8 and CD4% having the highest specificities, with overlapping confidence intervals.

Table 9

Comparison of Specificities and their 95% Confidence Intervals in Subjects Assigned Ritonavir

	CD4	95% CI	CD4%	95% CI	CD8	95% CI	CD8%	95% CI	Viral Load	95% CI
Baseline	46.00	41.60 50.40	47.86	43.37 52.35	36.00	31.69 40.27	31.49	27.31 35.67	58.21	47.53 68.89
Week 2	39.70	35.02 44.30	32.75	28.22 37.28	36.20	31.58 40.88	42.32	37.55 47.09	61.40	50.00 72.72
Week 4	50.40	45.68 55.16	49.71	44.88 54.54	39.40	34.70 44.14	32.95	28.41 37.49	45.45	33.62 57.28
Week 8	55.90	51.16 60.64	50.00	45.15 54.85	45.40	40.55 50.23	33.43	28.86 38.00	46.43	34.75 58.11
Week 12	55.00	50.08 59.96	54.97	49.98 59.96	50.30	45.28 55.34	38.82	33.94 43.71	26.00	15.34 36.66
Week 16	61.20	56.32 66.14	63.61	58.69 68.53	61.90	56.92 66.88	64.24	59.34 69.14	23.08	12.99 33.17
Peak	51.90	47.41 56.35	42.97	38.45 47.49	48.70	44.15 53.25	32.03	27.77 36.29	40.68	29.49 51.87
$TAUC_{16}$	56.70	52.19 61.17	69.63	65.38 73.88	62.10	57.63 66.59	54.66	50.06 59.26	77.97	68.53 87.41
$TAUC_{40}$	58.50	54.09 62.99	57.05	52.49 61.61	59.30	54.76 63.80	32.12	27.82 36.42	61.02	49.91 72.13

* shaded cells are of surrogate marker responses whose model χ^2 did not reach statistical significance ($p \geq 0.05$)

Table 10

Comparison of Specificities and 95% Confidence Interval For Subjects Assigned Placebo

	CD4	95% CI	CD4%	95% CI	CD8	95% CI	CD8%	95% CI	VL	95% CI
Baseline	43.34	37.94 48.74	47.12	41.58 52.66	47.47	41.96 52.98	34.94	29.65 40.23	46.67	32.09 61.23
Peak	46.89	41.44 52.34	36.33	30.98 41.68	42.44	36.95 47.93	44.69	39.16 50.22	51.22	35.92 66.52
$TAUC_{1h}$	56.43	50.99 61.87	49.68	44.11 55.25	49.84	44.28 55.40	56.27	50.76 61.78	60.00	44.82 75.18
$TAUC_{10}$	52.50	47.03 57.97	50.32	44.75 55.89	51.77	46.22 57.32	56.91	51.41 62.41	60.00	44.82 75.18

Table 11

Comparison of Specificities and 95% Confidence Interval For All Subjects

	CD4	95% CI	CD4%	95% CI	CD8	95% CI	CD8%	95% CI	VL	95% CI
Baseline	45.11	41.97 48.25	47.39	44.18 50.60	44.65	41.98 47.82	33.00	29.98 36.02	29.46	21.01 37.90
Peak	45.77	42.60 48.94	48.20	44.96 51.44	49.64	46.40 52.88	33.38	30.32 36.44	32.00	22.86 41.14
$TAUC_{1h}$	51.26	48.04 54.48	53.96	50.54 57.28	50.93	47.65 54.21	58.82	55.59 62.05	30.30	21.29 39.31
$TAUC_{10}$	50.00	46.78 53.22	55.78	52.51 59.05	58.37	55.14 61.60	39.02	35.83 42.21	45.45	35.69 55.21

Conclusions

For subjects assigned to the active therapy in this study, after adjustment for baseline variables, the univariate analyses produced markers with higher specificity and encompassed more of the study population than any of the multivariate analyses. There was no consistent interaction between baseline and other surrogate marker measures.

The surrogate marker response with the highest specificity is the CD4% $TAUC_{16}$. This is approached by the specificities of the CD4, CD4%, CD8 and CD8% at week 16, and by the baseline viral load. In general, the 16 and 40 week TAUCs are as good as or better than the time period surrogate marker responses to week 12, and are superior to the peak responses. These analysis are limited by the small sample size of those with viral load measures.

For placebo-assigned subjects and all subjects considered together, the time period markers were not assessed. For all subjects, the TAUCs are of higher specificity than the baseline and peak responses. For placebo-assigned subjects, only the baseline CD4 was associated with the development of endpoints.

4.10. Question 2

What time period of which surrogate marker is most highly associated with the clinical outcome?

Clinical practice requires "real time" assessment of surrogate marker responses; in other words, we need to know what degree of change in a surrogate marker at a specific time point (in response to the initiation of therapy) is most associated with protection from the development of opportunistic illness in a specified period of time (in this case, 40 weeks)? As such, the peak response (which can only be determined retrospectively, once all the data points are available) cannot be used in clinical management. As well, the TAUCs are determined retrospectively, and by their very nature, would not be amenable to use in clinical practice. Therefore, the peak responses and TAUCs were not considered. In addition, both Canadian and American guidelines suggest reassessment of the patient within 12 weeks of initiation of therapy, and the standard of clinical care is to see patient quarterly. As such, the Week 16 response was excluded from consideration as the best surrogate marker response for clinical use. This leaves the Week 2 to 12 responses for the ritonavir-assigned subjects (Table 12).

Table 12

Specificities and 95% Confidence Intervals of Clinically Useful Surrogate Marker Responses

	CD4	95% CI	CD4%	95% CI	CD8	95% CI	CD8%	95% CI	Viral Load	95% CI
Week 2	39.66	35.02 44.30	32.75	28.22 37.28	36.23	31.58 40.88	42.32	37.55 47.09	61.40	50.00 72.72
Week 4	50.42	45.68 55.16	49.71	44.88 54.54	39.42	34.70 44.14	32.95	28.41 37.49	45.45	33.62 57.28
Week 8	55.90	51.16 60.64	50.00	45.15 54.85	45.39	40.55 50.23	33.43	28.86 38.00	46.43	34.75 58.11
Week 12	55.02	50.08 59.96	54.97	49.98 59.96	50.31	45.28 55.34	38.82	33.94 43.71	26.00	15.34 36.66

The specificities of the CD4 and CD4% are consistently superior to those of the CD8 and CD8%.

Although the confidence intervals are wide and overlap with many others, the highest specificities are seen with the week 8 and 12 CD4 responses, and week 12 CD4%. Overall, for clinical assessment, the best surrogate marker is the Week 8 CD4 response (specificity = 55.90%, 95% CI 51.16-60.64).

4.11. Question 3

What is the diagnostic performance of the clinical and overall surrogate marker responses of greatest specificity?

Clinical

For the clinical surrogate marker response of greatest specificity (week 8 CD4 response, 55.90%), the logistic regression equation is as follows:

$$\beta = -1.6894 - 0.0099*(\text{Wk8 CD4}) + 0.6155*(\text{past major OI}) - 0.1343\ (\text{present RTI use})$$

where

wk8 CD4 = CD4 at week 8 - Baseline CD4

past major OI:	0 = no past OI	1 = past OI
Present RTI use:	0 = no present RTI use	1 = present RTI use

The odds ratio, positive and negative predictive values, and positive and negative likelihood ratios were calculated (Tables 13, 14, 15a, 15b, page 55).

Table 13

Odds Ratio for Illness with Changes in Week 8 CD4 Response in Subjects Assigned Ritonavir

	Increase ≥ 10	Increase ≥ 25	Increase ≥ 50	Increase ≥ 100
Odds Ratio	0.91	0.78	0.61	0.37

Table 14

Positive and Negative Predictive Values for Week 8 CD4 Changes in Subjects Assigned Ritonavir*

	increase ≥ 10	increase ≥ 25	increase ≥ 50	increase ≥ 100
PPV	25.0	22.7	18.8	16.5
NPV	91.3	92.9	94.1	93.1

PPV = positive predictive value NPV = negative predictive value

* pre-test probability of OI for those assigned ritonavir = 16.3%

Table 15a

Positive and Negative Likelihood Ratios for Week 8 CD4 Changes in Subjects Assigned Ritonavir

	increase ≥ 10	increase ≥ 25	increase ≥ 50	increase ≥ 100
+LHR	1.89	1.66	1.38	1.12
-LHR	0.54	0.44	0.36	0.42

+LHR = likelihood ratio for a positive test -LHR = likelihood ratio for a negative test

Table 15b

Positive and Negative Likelihood Ratios for Week 8 CD4 Changes in Subjects Assigned Ritonavir

	increase ≥ 10	increase ≥ 25	increase ≥ 50	increase ≥ 100
+LHR	0.59	0.43	0.35	0.42
-LHR	1.81	1.68	1.40	1.12

+LHR = likelihood ratio for a positive test -LHR = likelihood ratio for a negative test

Overall

The best surrogate marker response overall is that of the 16 week TAUC, which has a specificity of 69.63%:

$g(x) = -2.2930 - 0.0866*(CD4\%TAUC_{16}) + 0.07306*$ (Past Major OI) $- 0.3313*$(Present RTI use)

where

$CD4\%TAUC_{16}$ = time-averaged CD4% change per week over the first 16 weeks (in CD4%)

past major OI:	0 = no past OI	1 = past OI
Present RTI use:	0 = no present RTI use	1 = present RTI use

The odds ratio, positive and negative predictive values, and positive and negative likelihood ratios were calculated (Tables 16, 17, 18a, 18b, page 57).

Table 16

Odds Ratio for Illness with Changes in $TAUC_{16}$ in Subjects Assigned Ritonavir

	Increase 100%	Increase 250%	Increase 500%	Increase 750%
Odds Ratio	0.92	0.81	0.65	0.52

Table 17

Positive and Negative Predictive Values for $TAUC_{16}$ in Subjects Assigned Ritonavir*

	increase ≥ 1.0	increase ≥ 2.5	increase ≥ 5.0	increase ≥ 7.5
PPV	57.0	79.7	96.2	98.7
NPV	59.4	31.4	11.9	4.1

PPV = positive predictive value NPV = negative predictive value

* pre-test probability of OI for those assigned ritonavir = 16.3%

Table 18a

Positive and Negative Likelihood Ratios for $TAUC_{16}$ in Subjects Assigned Ritonavir

	increase ≥ 1.0	increase ≥ 2.5	increase ≥ 5.0	increase ≥ 7.5
+LHR	1.40	1.16	1.09	1.03
-LHR	0.72	0.65	0.32	0.32

+LHR = likelihood ratio for a positive test -LHR = likelihood ratio for a negative test

Table 18b

Positive and Negative Likelihood Ratios for $TAUC_{16}$ in Subjects Assigned Ritonavir

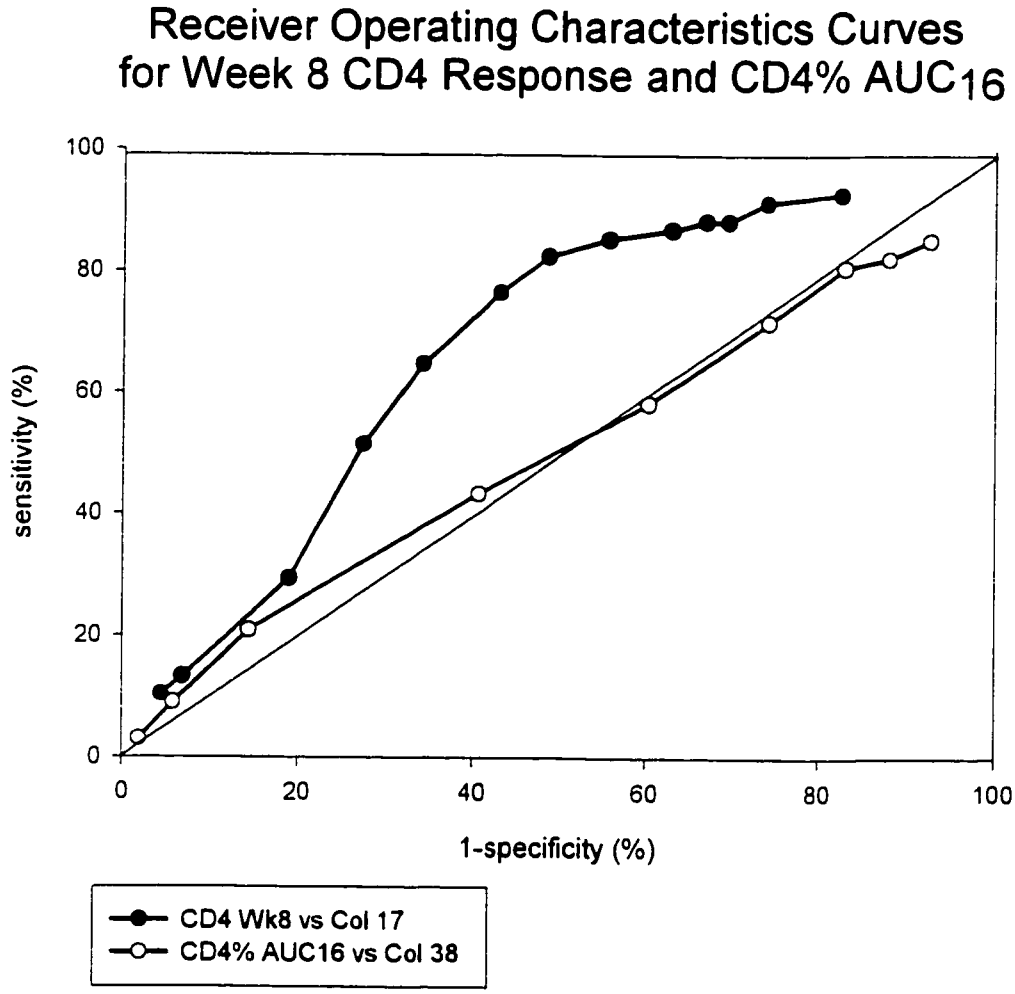
	increase ≥ 1.0	increase ≥ 2.5	increase ≥ 5.0	increase ≥ 7.5
+LHR	0.71	0.64	0.33	0.33
-LHR	1.40	1.17	1.11	1.03

+LHR = likelihood ratio for a positive test -LHR = likelihood ratio for a negative test

Receiver Operating Characteristic Curves

ROC curves were prepared for the time period (Week 8 CD4) and peak/TAUC surrogate marker (CD4% $TAUC_{16}$) responses of greatest specificity, for those assigned ritonavir (Figure 5). In summary, the ROC curve for the Week 8 CD4 response is clearly superior to that of the CD4% $TAUC_{16}$, although neither demonstrates adequate performance for that surrogate marker response.

Figure 5



Discussion

The correlation between surrogate marker response and clinical outcome is pivotal in the analysis of clinical trials in HIV, as well as many other illnesses. This has been examined, to an extent, in the literature, but mostly with older drugs (not protease inhibitors) and with peak or single time point responses examined. Interestingly, the Health Protection Branch (HPB) of Health Canada assesses surrogate marker responses as the time-averaged area under the curve , as does the American Food and Drug Agency (FDA). There is no formal assessment of this method of surrogate marker analysis in the literature.

The Abbott study M94-247 was the first study of single antiretroviral medication to demonstrate a survival advantage in some ten years. The study of over 1000 HIV-infected persons provides a great deal of surrogate marker and disease progression data, but is limited by the relative paucity of the viral load data (157 subjects). As well, the addition of a single drug to a therapeutic regimen, as was performed in this study, is longer considered the standard of care.

In general, the results demonstrate a greater validity of CD4 and CD4% as surrogate markers, as many of the other logistic regression equations for the surrogate marker changes from the CD8, and especially CD8% and viral load, are not of statistical significance. The specificity of surrogate marker changes increases with increasing time from treatment initiation. The peak surrogate marker responses perform poorly, whereas the $TAUC_{16}$ and $TAUC_{40}$ seem equivalent to, or superior to the time period surrogate marker changes. The surrogate marker change of greatest specificity was the CD4% $TAUC_{16}$ for the ritonavir-assigned subjects (69.63%). For subjects assigned placebo, the only surrogate marker to achieve statistical significance was the baseline CD4.

In no case was multivariate analysis of superior specificity to univariate analyses, and few significant interactions were found. The ROC curves confirm the rather poor performance of any of the surrogate

marker changes. As well, the odds ratios, the positive and negative predictive values, and the positive and negative likelihood ratios for the development of illness fell within a rather narrow range.

Presented below is further discussion of the methodology and the results.

5.1 Outcome Variable

The outcome variable of interest was chosen to be the occurrence of opportunistic illness. Other options would have included death, and the combined endpoint of opportunistic infection and death. Certainly, the occurrence of opportunistic infections in this study is a reasonable endpoint, as these were monitored by blinded external reviewers and required the fulfillment of standardized criteria for acceptance. As well, by definition, the occurrence of opportunistic illnesses is related to the presence of HIV-related immune deficiency; the reduction in this endpoint by the activity of an antiretroviral medication is biologically plausible. The occurrence of an OI may lead to significant morbidity and many are lethal; therefore, this endpoint is clinically reasonable. However, not all opportunistic illnesses are of equal severity, and some (esophageal candidiasis, limited cutaneous Kaposi's Sarcoma) are rather minor, easily induced (by discontinuation of secondary prophylaxis in the case of esophageal candidiasis) and easily treated. This allows for the manipulation of study endpoints in order to gain access to the study drug.

Death, as an endpoint, has several advantages. It is well-defined and obvious. It was relatively common in this study, having occurred in 60 subjects (without a prior opportunistic illness during the study) by week 40. It can occur in the absence of defined opportunistic illness but still be related to HIV (such as with HIV cardiomyopathy, or as a result of an illness whose treatment or natural history is altered by HIV, such as mental illness). If there are potentially lethal side effects of therapy, death as an endpoint may offer a more balanced assessment of drug usefulness. However, death may be unrelated to HIV infection or the degree of immune deficiency; it may be accidental, or the result, for instance, of unrelated medical problem or suicide.

The combined endpoint of death or opportunistic illness is commonly used as an endpoint. The pros and cons are as discussed above. There is the additional benefit of increased number of endpoints. There are concerns as to the inequality of the clinical illnesses possible with this endpoint. As well, the use of the combined endpoint would be clinically meaningful: physicians, with their prescribed therapies, try to keep patients alive longer and free of illness. However, there is a large difference between death and the occurrence of esophageal candidiasis, which may cause two or three days' worth of discomfort. In addition, death may not be related to HIV or the level of immune dysfunction. There remains, as well, the possibility of manipulation of the endpoints by medication discontinuation.

Because of the comparatively small number of deaths in this study and the possibility that some could be unrelated to HIV, death as the primary outcome was rejected. The potential large inequality of endpoint severity and the possibility that some deaths were unrelated to HIV or ritonavir was responsible for the rejection of the combined endpoint of death or opportunistic illness. The development of opportunistic illness was chosen not only by exclusion, however; it was felt that this would best reflect ritonavir's anti-HIV activity, and was clinical relevant and meaningful.

It was decided to look primarily at those assigned active therapy, with the placebo-assigned subjects and the entire cohort as secondary analyses to assess generalizability of results. Examination of placebo-assigned subjects would look at those on stable therapy, as well as those changing RTI therapy after 16 weeks. Analysis of surrogate marker responses among those assigned to therapy would be relevant to clinical decision making in the context of initiation of therapy. It would allow for determination of at what time point measurement of which surrogate marker would be most associated with clinical benefit of the initiated therapy. However, analysis of the responses of the entire cohort and placebo subjects may be more generalizable, and may offer insights into disease pathogenesis. For all subjects, is a 50 cells/μL CD4 increase associated with clinical outcome? Is this less predictive than for those assigned to therapy, implying that random fluctuations are less important clinically than treatment-induced CD4 (or other surrogate marker) changes?

It was decided to examine the relationship between absolute surrogate marker change (as opposed to percent change) from baseline and opportunistic illness. The risk for opportunistic illness is certainly greatest with extremely low CD4 cell counts; the median CD4 cell count for many opportunistic illnesses, or death is less than twenty[19]. As such, a twenty cell CD4 rise from this range may be of relatively greater clinical benefit than an identical rise from a CD4 cell count of 100. There are equal or greater difficulties with the use of percentage increases. There may be a discrepancy between the clinical benefit of a 50% CD4 cell rise from a baseline of 2 cells/μL as compared to the same proportional increase from a baseline of 30 cells/uL. There are also practical difficulties. Baseline surrogate marker values of zero are present in the database; for instance, ten subjects had a baseline CD4 cell count of zero. It would be impossible to calculate a percent change from zero. As a result, absolute surrogate marker changes were used.

5.2. Baseline Variables

Baseline variables were chosen for consideration of inclusion into the model based on the scientific plausibility of their association with the outcome of opportunistic illness, particularly if there was epidemiologic or clinical trials evidence otherwise of such a relationship.

Past Opportunistic Infections

In the case of past major or minor opportunistic infections, these (and particularly the former) likely indicate more advanced immune deficiency, with a lower CD4 cell count and possibly increased viral load. These are demonstrated risk factors for the further occurrence of opportunistic illnesses[71]. HIV-related opportunistic infections are more common in those with oral candidiasis[71,72], oral hairy leukoplakia[73], and herpes zoster[74,75]. Second episodes of opportunistic infections are more common after the first: the six month risk of *Pneumocystis carinii* pneumonia (PCP) for those with a CD4 cell count of <200 cells/μL and no history of OI was 8.4% in a large study of 1665 HIV-infected homosexual men[71], whereas the annual risk was 65% for those with a single past episode[14].

The frequency of past major OI in the placebo and ritonavir groups was 61.3% and 67.1%, respectively (χ^2=1.344 with df=1; p = 0.246). In our analysis, the past history of major opportunistic illness (which occurred in 69.7% of evaluable subjects) was found to be the baseline variable most strongly associated with the endpoint. The OR of 1.4741 for all subjects, and 2.0705 for those assigned to ritonavir (where the endpoint is that of the development of an opportunistic illness) suggests that the past history of major opportunistic illness is, in fact, associated with a greater risk of developing an endpoint in this study. This is consistent with other studies, and may prompt the treating physician to treat with more aggressive antiretroviral regimens, and to provide appropriate and effective prophylactic measures for opportunistic infections (trimethoprim-sulfamethoxazole for PCP prophylaxis, vaccination for influenza and pneumococcal pneumonia, etc).

Why does the past history of major OI increase the risk for further OI? The occurrence of an OI may be a marker for a low CD4 cell count, which is the greatest risk factor for further OIs. In this study, the mean CD4 cell count ± SD for those without past OI and those with past OI was 39.47 ± 30.10 and 26.29 ± 25.02, respectively (F = 50.238, p < 0.001). As well, OIs are more common with higher viral loads; in this study, the baseline viral load of those with a past major OI was not significantly higher than those without (5.18 vs 5.26 $\log_{10}$ copies/mL, F = 0.523, p = 0.471).

The lack of association between minor opportunistic illness and development of clinical endpoints in this study is not surprising in spite of the association demonstrated in other studies. Those enrolled in this study had advanced HIV, with a CD4 count below 100. It is to be expected that most subjects had previously suffered a minor opportunistic illness; in fact, 900 of 1000 evaluable subjects in this study had previously had a minor OI. Therefore, the frequency of this occurrence would render it poorly discriminatory with regards to future illness.

Reverse Transcriptase Inhibitor Use

Present RTI use is likely of importance in terms of the magnitude and duration of virologic and clinical

response to ritonavir. There is ample evidence that reverse transcriptase inhibitor monotherapy is superior to placebo in terms of prevention of HIV-related illness or death[13], and that up front double therapy is superior to monotherapy[15,76]. The virologic and immunologic benefit of protease inhibitors is prolonged with concomitant effective RTI therapy[77]; in this context, it is likely that any beneficial effect of the study drug would be multiplied if there was concurrent effective RTI therapy, and blunted if ineffective (monotherapy) or no therapy was used.

In this study, the present use of any RTI was of borderline statistical significance for all subjects, as well as those assigned to ritonavir. The odds ratio of 0.9455 for all subjects, and 0.6510 for those assigned ritonavir, suggests that the concomitant use of any RTI(s) in this study is associated with a reduced likelihood of the subsequent development of OIs. The lower odds ratio for those assigned to ritonavir suggests that there may be synergy between these drugs. Ongoing RTI use is more protective from opportunistic illnesses when used with ritonavir; this is in keeping with clinical trials evidence of improved degree and durability of antiretroviral response of protease inhibitor-RTI combinations than RTIs alone. The present standard of care is the use of two RTIs with one or two protease inhibitors.

Added Reverse Transcriptase Inhibitors

In this study, the addition of reverse transcriptase inhibitors before 16 weeks or at any time to 40 weeks was not associated with the development of clinical endpoints. Past studies have assessed the effect of the addition of a single RTI to ongoing RTI therapy (as opposed to up front double RTI therapy in treatment-naive persons, as described above). A large CPCRA study[79] documented no survival benefit with the addition of didanosine or zalcitabine to ongoing zidovudine therapy in persons with advanced HIV and a past history of zidovudine use. The reasons for this are likely several. Concerns would include cross-resistance between RTIs, such that virologic resistance to one would be associated with poor response to another. As well, the addition of a single RTI to failing antiretroviral therapy (in which the first RTI is *de facto* not present, because of the development of

viral resistance) is akin to monotherapy: the use of a single drug. This is of limited and transient virologic and immunologic benefit and would not likely have significant effects on morbidity or mortality. In light of the above, and with knowledge that the study subjects had advanced HIV and were therefore likely to have received many RTIs in the past (and therefore have highly resistant viral strains, or not have the opportunity to add two new RTIs, which is likely superior to adding only one), it is not surprising that RTI addition was not of any clinical benefit. Present guidelines do not suggest the addition of single RTIs to a failing regimen.

Age

Age has been found to be a predictor of survival in HIV[78], with those of increased age *at the time of initial HIV infection* having a reduced survival. It is thought that this may be related to reduced CD4 proliferative capacity in those with increased age, as a result of reduced chromosomal telomere length; this would lead to a more rapid rate of CD4 decline and therefore earlier development of OIs. However, as expected, age was not associated with outcome in this cohort, as the patients have already progressed to the advanced stages of HIV infection and are at high risk for illness; the rate of further decline of immune function would be of little importance.

It may be that the replicative capacity of CD4 cells is reduced with increased age; even though the virologic effect of ritonavir may not change with age, the CD4 cell increase may be blunted in older individuals. This has not been addressed in previous studies. There is no such association in this study, as, for those assigned ritonavir, there was no correlation between maximal CD4 increase and age with either 16 weeks $r = 0.036$, $p = 0.42$) or 40 weeks $r = 0.04$, $p = 0.80$).

Baseline Variables Not Tested: SI/NSI Phenotype and Compliance

Important variables which may be significantly associated with clinical outcome are the SI/NSI HIV phenotype, and medication compliance. The former is an *in vitro* phenomenon in which HIV with the SI phenotype causes the fusion of mononuclear cells. How this viral phenotype exerts its influence *in*

vivo is unknown, but its presence has been consistently associated with increased progression to AIDS and death in clinical studies[68,69,70]. HIV phenotyping was not available from the study sponsor.

Compliance with antiretroviral therapy has been correlated with virologic and immunologic response, and survival[80]. This is likely more important for protease inhibitors, like ritonavir, than reverse transcriptase inhibitors, like AZT, because of the shorter plasma and intracellular half-life of the former. Reduced compliance would lead to lower drug levels, which would predispose the patient towards the development of resistance and therefore loss of effect. Unfortunately, compliance data was not available from the study sponsor.

5.3. Covariates

5.3.1. Statistical Issues

The covariates examined, the CD4, CD4%, CD8, CD8% and viral load, are standard measures in clinical practice. Prior to the development of viral load testing, other commonly used tests would have included the p24 antigen, serum neopterin, and β2-microglobin. These markers were not measured in this study.

CD4 and CD8 cell counts are provided through direct measurement, which, along with other T lymphocytes, provides the total lymphocyte count. It is from this that the CD4% and CD8% are calculated. The correlation between baseline CD4 and CD4% is high (Spearman's coefficient: 0.757, $p < 0.001$); the correlation is much lower between CD4% and the baseline CD4 divided by the sum of baseline CD4 and CD8 (Spearman's coefficient:0.069, $p = 0.029$). This suggests that the CD4% is not merely a transformed version of the CD4, and is amenable to statistical analysis in its own right. The same is true of the CD8%. In spite of these degrees of relatedness between the CD4 or CD8 and the corresponding percent, significant statistical collinearity was not seen with the multivariate analyses. Nonetheless, clinical experience demonstrates that the CD4 and CD4% move together in

response to treatment, as do the CD8 and CD8%: they are clinically collinear.

The 16 week TAUC was studied to look at the response to the study drug alone. In this time period, study subjects were not to have altered antiretroviral therapy (although some did); therefore, the TAUC should model for slope, magnitude and duration of surrogate marker change in response to assigned therapy. The TAUC for the entire 40 week period of observation was calculated to look at the response to the therapeutic approach (be that through the addition of therapy or not, both before and after the 16 week cutoff). The 40 week TAUC should assess the clinical response to surrogate marker changes in general, be they related to antiretroviral therapy, random changes in surrogate marker levels, or otherwise.

It had been intended to evaluate the association of Tmax (time to peak surrogate marker response) and Tmin (time to return to baseline of surrogate marker response) with clinical endpoint in this study. However, this could not be performed in this analysis because of the variability of individual surrogate marker response curves. There is a large amount of biologic and measurement variability of both viral load[33] and CD4 cell count[23,24]. As a result, there are often decreases in immunologic markers (or increases in viral load) early in the study, even among those assigned to the ritonavir arm. This leaves the potential for:

1. Return to baseline (or decrease below baseline) at the first point of follow-up.
2. Return to baseline before (and therefore precluding) peak response.

Because of the perceived inappropriateness of the data for this type of analysis, the variables of Tmax and Tmin were not evaluated.

It would be expected that many of the covariates would not be normally distributed. For instance, the rate of CD4 decline is rapid with CD4 cell counts below 400[28], and death does not invariably occur

quickly once the CD4 count approaches zero. As such, in this study of patients with advanced HIV, the distribution of baseline CD4 measures are skewed towards zero. As well, the distribution of the CD4 cell count changes after baseline for all subjects, including the peak/AUC parameters, would not be expected to be normally distributed, as they represent two distinct populations: those assigned to ritonavir, and those assigned placebo. As expected, graphing of the covariate values suggested a lack of normality for most variables (data not shown). Further exploration of the surrogate marker data suggested non-normality ($p < 0.05$ with Kolmogorov-Smirnov One-Sample Test) of a large number of the variables. However, normality of distribution is not required for the performance of logistic regression, as no assumptions are made about the distributions of covariates with this technique.

The final important point regarding the covariates is that of sample size. Although 1090 subjects were randomized into the study, only 159 were to have had viral loads measured (although a total of 170 actually have baseline measures). Of these, 86 were subjects assigned ritonavir, and 15 developed an OI during the study period. This small sample size greatly limits the power of analyses involving the viral load. The small number having viral load measures likely reflects the early stage of this technology at the time of study initiation, and its expense (>$100.00 per test).

The sample size and number of outcomes otherwise are large, and the power was likely adequate to determine whether associations between the surrogate marker response and outcome were present. This study remains one of the largest HIV therapy trials to date, and the number of outcomes among the highest.

5.3.2. Results

Univariate and Adjusted Analysis

Comparison of specificities derived from adjusted and unadjusted analyses reveals several trends. There is no consistent effect of the baseline variables on the specificity of the CD4 or viral load

response for either the entire study population or those assigned to ritonavir. However, there is a generally negative effect of the baseline variables on the time period surrogate marker changes, especially the CD8 and CD8%, which, without adjustment, are highly specific.

The effect of the two baseline variables on the surrogate marker responses could be complex. As previously described, the past occurrence of a major opportunistic illness has a well described association with further clinical illness; as well, it might be associated with a reduced baseline CD4 count or increased viral load, and therefore perhaps a reduced magnitude or duration of antiretroviral response[71]. The ongoing use of RTI therapy might be expected to increase the magnitude or durability of therapy, and be therefore reduce the development of illness[77]. This would likely incur a positive bias: it would increase the specificity. Alternatively, RTIs are, to an extent, myelotoxic[81]; that is, they may suppress the bone marrow. This may lead to a reduced potential for appropriate CD4 response to therapy, or may lead to other conditions (such as neutropenia) that predispose to opportunistic illness. They may render other medications less tolerable; a subject may be less able to take specific opportunistic infection prophylaxis (such as trimethoprim-sulfamethoxazole for the prevention of *Pneumocystis carinii* pneumonia) while taking RTIs, and therefore be more susceptible to opportunistic infection. The effect of these on surrogate marker responses may differ from marker to marker.

Examination of the LLRχ^2 demonstrates that many of the covariates are not associated with the outcome of opportunistic illness to a statistically significant degree. The LLRχ^2 represents the difference in log-likelihoods between models with and without the covariate of interest. This is represented as:

$$G = -2 \ln \left(\frac{\text{(likelihood without the variable)}}{\text{(likelihood with the variable)}} \right)$$

Under the hypothesis that the covariate of interest is equal to zero, the test statistic G will follow a chi

square distribution with one degree of freedom. As such, a non-significant model χ^2 indicates that the covariate does not explain significantly more of the variability of the dependent variable than the equation without that same covariate.

Baseline Surrogate Marker Values

For all subjects considered together, the baseline viral load was not significantly associated with the endpoint of clinical illness. This held true for those assigned to receive placebo as well. This is in contrast to studies that have demonstrated that both CD4 and viral load are associated with risk of illness: lower baseline CD4 cell count and increased viral load are associated with an increased risk of illness in a cohort, and the risk of illness increases with higher viral loads for any CD4 cell count range.

For those assigned to receive ritonavir, higher baseline viral loads were associated with a significant increase in the risk of illness development. Very large increases in risk of illness are seen with increasing baseline viral loads above the lowest value in this study cohort (Table 19):

Table 19

Odds Ratio for Illness with Increasing Baseline Viral Loads in Subjects Assigned Ritonavir

	≥ 0.5 $\log_{10}$	≥ 1.0 $\log_{10}$	≥ 2.0 $\log_{10}$
Baseline	2.28	5.21	27.10

This may be explained by the association between higher viral loads and lesser degrees of viral load suppression. In this study, those with higher baseline viral loads had significantly higher nadir viral loads ($r = 0.457$, $p < 0.001$). Other studies[82] have demonstrated a strong association between nadir viral load and duration of suppression. Therefore, those in this study with higher viral loads pre-treatment may have responded to ritonavir for a shorter period; this may have led to a shorter duration of clinical benefit and therefore higher risk of illness.

Time Period Surrogate Marker Changes

A large number of covariates are not significantly associated with the endpoint of clinical illness. For those assigned ritonavir, this is particularly true of CD8% and viral load. Baseline and time period changes in viral load have been associated with the development of clinical illness in many other studies; in this one, the analysis may be hindered by the small number of subjects. A more sensitive technique, like Cox Proportional Hazards Modeling, may be necessary to assess this properly. There is no published data of the utility of CD8% measures in prognostication in HIV; as the sample size is large, it is likely that little true association between the level of change in CD8% and clinical outcome exists.

For all the adjusted variables, the week 2 surrogate marker responses were not significantly associated with the outcome. It is evident that there is a very strong monotonic linear trend towards increased specificity for all the surrogate markers, except for the viral load, with increasing time after study initiation. In three of four surrogate markers, this is more prominent for those assigned to ritonavir. This trend may be the result of selection bias: those with poor surrogate marker responses but who did not to become ill may have been lost to follow-up (as specificity = true negatives ÷ (true negatives + false positives), where true negatives are those classified as not to become ill and do not, and false positives are those classified as likely to become ill (because of poor surrogate marker response) but who do not. In other words, those with poor viral load or CD4 responses may have been preferentially lost to follow-up.

It should be noted that, to some extent, the time points chosen for immunologic and viral load monitoring are arbitrary: there is no biologic reason why these should be measured at two weeks as opposed to three, for instance. However, measurements at four, eight or twelve weeks have become, to an extent, the standard of care[20,21]. It may be that measurement of the specificity of surrogate marker responses at other time points may yield significantly different specificities; however, the consistent monotonicity of the surrogate marker specificities suggests that this is unlikely.

Time period surrogate marker responses (week 2 to week 16) for placebo assigned subjects are of little relevance for this study, as they do not represent time points after initiation of therapy; rather, they are time points after which they were entered into a study without any significant change in their clinical management. Therefore, time period surrogate marker responses were not assessed. For those assigned placebo, there was no significant association between the development of clinical illness and peak, $TAUC_{16}$ or $TAUC_{40}$ for any of the surrogate markers. The only covariate associated with the outcome was the baseline CD4 cell count. Several comments about these findings should be made:

1. The baseline CD4 has been associated with the development of clinical illness in a number of previous studies of both the natural history of HIV and the response to treatment. However, the viral load has been associated with the development of illness in many studies as well; it may be that the sample size is too small for this association to reach statistical significance.

2. The lack of significance of the peak or TAUC surrogate markers implies that short term changes in surrogate marker levels unassociated with new therapies are not associated with changing risk for opportunistic illness.

Assessment of the entire cohort increases the sample size, but, as assessment of the placebo subjects mostly yielded non-significant results, would be expected to reduce the specificity of most of the results. Comparison of the ritonavir-assigned subjects to all subjects reveals an inconsistent relationship between corresponding surrogate marker responses. Surprisingly, the CD4% TAUC16 is dramatically lower for all subjects than the ritonavir-assigned ones, and the TAUCs, for the most part, are reduced in specificity.

The improved performance of the CD4 in those assigned to ritonavir, as compared to placebo or all subjects, may have several explanations. Most importantly, fluctuations of the CD4 or CD4% in the absence of effective treatment may be of little clinical significance and therefore not alter the

specificity. As documented elsewhere[23,24], there is significant biologic and measurement-related variability of the CD4 cell count. The use of a potent antiretroviral, like ritonavir, may reduce that variability and make evident a relationship previously insignificant. A similar effect may occur with the viral load.

Peak/AUC Surrogate Marker Changes: Statistical Considerations

The TAUC was examined as a possible covariate with the expectation that it would represent a time modeled variable, talking into account magnitude and duration of surrogate marker change. Both were calculated with the equation

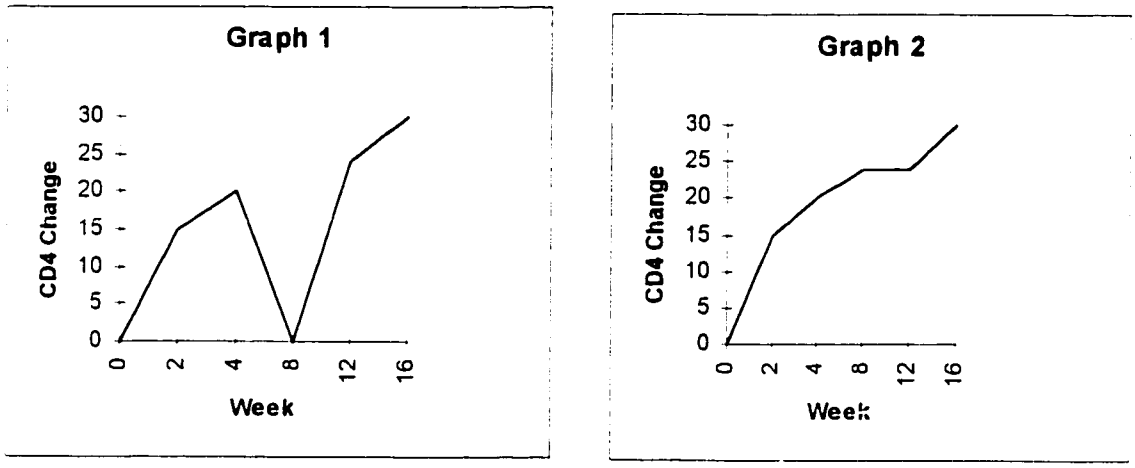
$$TAUC = \Sigma \frac{(a + b)\, h}{2}$$

where "a" and "b" are the vertical changes in surrogate marker responses at the start and end of a defined time period, and "h" is the length of the time period. The TAUC used in this study is not a complete TAUC, as there is no extension of the surrogate marker curve to the end of the time period of interest. In a pharmacokinetic analysis, for instance, complete curves are generated to determine (or extrapolate) peak and trough drug levels. This was not performed in this study.

There is the potential problem of missing data: use of this formula would lead to an inaccurate calculation of the TAUC, as the missing data point would be counted as a "0" by the SPSS program [Graph 1], as opposed to the use of the next data point and appropriate adjustment of the "h" [Graph 2]. This is demonstrated graphically in Figure 7 on the following page:

Figure 6

Illustration of the Correction for Missing Data



Subjects with missing baseline values were excluded from the analysis, as no change from baseline could be calculated. Otherwise, missing data points were extrapolated as being in the midpoint between adjacent values. For multiple consecutive missing points, their values were calculated as being on a straight line between adjacent available data points.

This method of accounting for missing data assumes a constant slope between adjacent known surrogate marker values. Almost certainly, this is not the case in reality. Bias could have been introduced with this method, if those with HIV-related illnesses may have missed appointments (and therefore surrogate marker measurements) because of illness (but before diagnosis). For instance, *Pneumocystis carinii* pneumonia or *Mycobacterium avium* complex infection may cause weakness, fever and anorexia for weeks prior to diagnosis. As well, as these illness may suppress CD4 cell counts and increase viral loads[34], the surrogate marker TAUCs of those with these opportunistic illnesses may be overestimated by my method. These factors would act to reduce the sensitivity of the response.

As well, this method of missing data estimation may overestimate the TAUCs of those assigned to placebo. Subjects who became ill (more likely to have been assigned placebo) are more likely to have

missed outpatient appointments for surrogate marker measurement, as they might have been in hospital or otherwise unavailable. Again, illness tends to decrease CD4 cell counts and increase the viral load; the method of adjusting for missing data may overestimate these.

For calculation of the TAUC to the time of an endpoint (or, in this analysis, to the study visit before endpoint development) when data is missing immediately prior to the pre-endpoint visit, the last available surrogate marker measure was carried forward to the time pre-endpoint visit. As above, the increased likelihood of illness in the placebo group might translate into more missed clinic visits, and therefore a larger proportion of placebo subjects having this type of data extrapolation performed than those assigned active therapy. However, as fluctuations in surrogate marker responses can occur in either direction, it may be that no consistent over- or underestimation of the TAUCs resulted. As above, though, cellular responses may be suppressed and viral load increase by opportunistic illness: therefore, the sensitivity may be overestimated.

Another source of bias might be related to the lack of blinding of surrogate marker responses. Those subjects with good responses (likely to be randomized to the ritonavir arm) would be expected to be more compliant with follow-up, as they were receiving a highly active, otherwise unavailable drug. This would provide a more exact calculation of the $TAUC_{16}$.

In addition to the missing data, there were the problems of different periods of follow-up (as many study visits were lost to follow-up because of illness, death or non-compliance) and irregular periods of follow-up (as clinical illness usually occurred between study visits). To adjust for these problems, a time-averaged TAUC was used to compare the TAUCs of those who did not develop an endpoint as compared to those who did; the data was censored at the last time point at which surrogate marker data was available for the former, and at the point of illness for the latter. However, this point of illness may not be at a study visit; for instance, it may be at day 32, which is four days after the week 4 visit. We considered the use of an exact analysis, necessitating calculation of the projected TAUC based on that

of the previous interval. For instance, consider the case of an endpoint occurring four days after the week 12 visit. The exact analysis would added 4/28 of the TAUC calculated between weeks 8 and 12 to the total TAUC. However, this method of analysis was rejected because of the required amount of work for the calculations, and the high degree of correlation (Pearson $r=0.902$, $p<0.001$ for the CD4 cell count for all subjects) with the so-called truncated analysis, in which calculation of the TAUC was carried forward only to the study visit immediately preceding the endpoint. As such, the truncated analysis was carried out.

The use of the time-averaged TAUC adjusted for unequal lengths of follow-up, reducing this type of bias. However, others were introduced. In particular, the TAUC for those who developed endpoints is somewhat underestimated, as the entire period of observation is not used because of the truncation. Assuming that the likelihood of illness is, in fact, related to the magnitude and duration of surrogate marker response, the lower TAUCs of those who become ill should in fact increase the sensitivity of the surrogate marker responses.

The ideal study for the proper assessment of PK parameters would have been one in which there was no provision of open label ritonavir after development of an opportunistic illness. One could therefore look at the full surrogate marker curves over the 16 or 40 week period, without need for correction for new drug addition.

Peak and AUC Surrogate Marker Changes: Results

The specificities of peak surrogate marker responses were inferior to corresponding best surrogate marker time period changes, and all are inferior to the week 8 CD4 changes (although the 95% CI of the week 8 and peak CD4 responses overlap). Therefore, in this study, there is no benefit of looking at peak CD4 responses as compared to the standard week 8 or week 12 CD4 responses.

The assessable 16 and 40 week areas under the curve compared favourably with to the time period

variables. With the exception of the CD8% $TAUC_{16}$, all exceed the best surrogate marker response to week 12. The best PK surrogate marker response is the CD4%$TAUC_{16}$ (specificity = 69.63%, 95% CI 65.83, 73.88); its 95% CI does not overlap with those of the other PK responses or time period responses, with the exception of the Week 16 responses for CD4, CD4%, CD8 and CD8%. Again, the week 16 measures may partly reflect loss to follow-up of treatment failures, and therefore are biased and of limited generalizability.

Multivariate Analysis

Other studies have demonstrated that the CD4 cell count and viral load may offer independent and additive value as surrogate markers. For instance, the risk of opportunistic illness or death has been demonstrated to increase with increasing levels of viral load for a given CD4 cell range[40]. As such, multiple logistic regression was carried to determine whether a combination of covariates at any time point, or a combination of PK parameters, would lead to improved specificity. Concerns could be raised about the potential for significant statistical collinearity because of the large degree of correlation between some variables. However, only five of the seventy-two assessed multivariate models contained more than one covariate, and in none of these was the standard error unusually large. As such, no problems with collinearity were encountered.

In this study, multivariate analysis entered the significant baseline variables of present RTI use and past major opportunistic infection, with stepwise regression of the covariates of interest, to determine if there a combination of variables would lead to significantly greater specificity than one alone. Only covariates from a single time period were entered together, as responses of the same surrogate marker from different time points would not be independent. These analyses were performed with several patterns of covariates entered:

1. All five surrogate markers entered. This allowed for all potential interactions to be assessed, but was hindered by the small sample size, due to the small number of subjects that had viral load testing

performed. Of the study cohort of 1086 subjects, only 170 had a baseline viral load drawn. For those assigned to ritonavir, only 86 had a baseline viral load, and of these 86, only 15 experienced an endpoint.

2. Only CD4, CD8 and viral load entered. Again, this was hindered by the small sample size.

3. All surrogate markers except for the viral load. This limits the number of interactions, and precludes examination for the one of greatest interest (the interaction between CD4 cell count and viral load). However, the sample size is much larger than for the previous two analyses.

4. All surrogate markers except viral load, but including only the subjects for whom viral load measures were available. Comparison of these results with those of #3 allowed for evaluation of the effect of the sample size on this type of analysis.

In analysis one, as described above, only at week 8 among all subjects was more than one variable included. This, however, yielded a specificity of only 40.00%. Several specificities among the ritonavir-assigned subjects were in excess of 50%; the baseline equation incorporates only the viral load, and as such is identical to the univariate equation. The other two, the week 16 and the $TAUC_{40}$ equations, incorporate only one variable and have very small sample sizes.

The situation for the second type of analysis is similar to that described above.

The third type of analysis excludes the viral load and therefore has larger sample sizes. Again, most of these incorporate a single covariate, usually the CD4. As such, even though the specificities are higher than in the previous two, and as high as or exceeding those of the univariate CD4 analyses, this is likely related to the smaller sample sizes than the univariate analyses and a resulting degree of bias.

The fourth analysis, similar to number three but using only data from the subjects having had viral load measures, reveals that the specificities in general decrease with this marked reduction in sample size. As well, the CD4 is less well represented as a chosen covariate.

Interactions

It is not unexpected that interactions might occur. These were only investigated for subjects assigned to ritonavir. There has been no published data on the effect of baseline levels of CD4, CD4%, CD8 or CD8% on subsequent treatment-related changes. However, it is reasonable to assume that these cellular responses will be blunted if there are few CD4 cells left to respond to treatment; whatever causes the cells to deplete in the first place may also limit their potential for recovery. Having said this, our clinical experience has been that HIV-infected persons with very low CD4 cell counts may respond to therapy with increases to 400-600 cells/μL.

As well, higher baseline viral loads might be associated with the more rapid development of resistance with sub-optimal drug levels, or the pre-existence of drug-resistant HIV quasi-species. These might limit the magnitude, and especially the duration, of effect of therapy.

No significant interaction was detected between baseline CD4 or CD4% with subsequent treatment-related changes, implying similar degrees of surrogate marker change regardless of baseline CD4 cell count. The baseline CD8 and CD8% interacted significantly with the corresponding $TAUC_{16}$; the explanation for and clinical significance of this are uncertain. Very early and peak viral load responses interacted with the baseline level, and the week 8 interaction approached clinical significance; although suggestive that many viral load responses will interact with the baseline level, the small sample size limits the ability to investigate this further.

Assessment of the degree of interaction between baseline viral load and baseline levels of the other surrogate markers is also limited by the small number of subjects having had viral load determinations

performed. Three of the interactions have a *p* value below 0.20, one (CD8%) below 0.10. Nonetheless, none reach the *p* value of 0.05.

5.4. Clinical Interpretation

As described above, the increasing specificity over time of the surrogate marker responses is not unexpected, and is likely related to selection for persistent surrogate marker responses. The other important finding with regards to clinical utility of these responses is the superiority of the CD4 and CD4% responses over those of the CD8 and CD8%; unfortunately, the viral load could not be assessed adequately because of the small numbers that had this done.

For the purposes of clinical management of patients, who would typically be re-assessed within twelve weeks of medication initiation, the best surrogate marker responses (in terms of specificity) would be the week 8 or 12 CD4 response, or week 12 CD4%. There is significant overlap of the 95% confidence intervals of these responses.

Regarding the best time period, the week 8 CD4 response of those assigned ritonavir has a specificity of 55.90%, indicating that 55.90% of subjects in this study who were free of opportunistic illness in the 40 week period were correctly predicted to be free of illness by the equation:

$$\beta = -1.6894 - 0.0099*(\text{Wk8 CD4}) + 0.6155*(\text{past major OI}) - 0.1343\ (\text{present RTI use})$$

where

wk8 CD4 = week 8 CD4 response (= CD4 at week 8 - Baseline CD4)

past major OI:	0 = no past OI	1 = past OI
Present RTI use:	0 = no present RTI use	1 = present RTI use

From this equation, one can develop odds ratios for the likelihood of illness development (compared to no CD4 change) with incremental changes in CD4 (assuming no past major OI and no present RTI

use). Table 13 (page 55) demonstrates that an increase in CD4 cell count of 100 at week 8 is associated with an odds ratio of 0.37 for the development of illness, as compared to no change in CD4 at that time period. This is in keeping with the expected response to improved CD4 cell counts: they should be associated with improved immune status and reduced clinical illness.

For the week 8 CD4 change, the coefficient for past major OI, being positive, indicates that the presence of a past OI (coded as "1") is associated with increased risk of OI in the study period, whereas the negative coefficient for present RTI use suggests these drugs are protective from illness.

For the week 8 CD4 response, the positive and negative predictive values for selected changes are illustrated in Table 14 (page 55). This demonstrates that 22.7% of those with a CD4 increase of less than or equal to 25 cells/μL at 8 weeks will experience an opportunistic illness by week 40. Conversely, 92.9% who have a CD4 increase of *greater than* 25 cells/μL at week 8 will remain free of illness in the 40 week time period of this study. Furthermore, the PPV and NPV of CD4 increases of ≥25 cells/μL would be 7.1 and 77.3, respectively; this implies that 7.1% of those with a CD4 increase of 25 cells/μL at 8 weeks would become ill in the 40 week period, whereas 77.3% of those with CD4 increases less than 25 cells/μL would remain free of illness in this time period. These compare with the pre-test likelihood (or 40 week prevalence) of opportunistic illness of 16.3% for those assigned ritonavir, and 23.8% fro all subjects.

For clinical purposes, this type of analysis is very useful. With a known surrogate marker change, one can determine what the likelihood of illness, or freedom from illness, would be within a specific time frame. It can be seen that the likelihood of illness decreases from 16.3% in 40 weeks to 6.9% if the CD4 increases 100 cells/μL after eight weeks. However, the NPV and PPV have limitations. They are highly dependent on the prevalence of the outcome illness; reduced prevalence of the outcome usually leads to a lower PPV and higher NPV. As well, they are of limited use when the outcome is of low prevalence, and most likely to significantly change the post-likelihood when the prevalence is 50%. As

well, these values are of limited generalizability. Being so highly dependent on the prevalence of the outcome, the values determined in this study would not be relevant to most other populations, especially with the rapid evolution of HIV treatment. Specificity and sensitivity, and likelihood ratios, which are calculated vertically, would be less so influenced.

The likelihood ratios for week 8 CD4 changes for those assigned ritonavir are illustrated in Tables 15 a and 15b (page 55). The +LLR of 1.55 for a ritonavir-assigned subject with a week 8 CD4 cell change of ≤ 0 cells indicates that the risk of illness is increased 1.55 times for those with less than or equal to 0 cell increase at this time point, compared to a cell count rise greater than this. It is expected that this likelihood ratio should decrease with increasing degrees of CD4 change. As well, as the second table indicates, CD4 rises of 0 cells or greater are associated with reduced risk of illness, and this decreases with greater CD4 cell count changes.

This may be the preferred clinical interpretation of the study results, as the likelihood ratio can be calculated for many levels of surrogate marker change, and is not dependent upon prevalence of the outcome: it is generalizable. The problem with their use is the requirement for knowledge of the pre-test likelihood of a disorder for determination of the absolute risk of illness: the likelihood ratio, when multiplied by the pre-test odds for a target disorder, yields the post-test odds for the disorder. Nonetheless, the relative likelihood of illness with surrogate marker changes in itself is valuable clinically.

Regarding those not assigned to ritonavir in this study, presumably random changes in the surrogate markers over time are demonstrated to be of little statistical association with the endpoint of opportunistic illness. More important might be a trend towards increasing viral load or decreasing CD4 cell count on present therapy, which has been demonstrated to predict a poor prognosis[57,62]; the diversity of therapies, presumably of differing potencies and durations, would preclude examination of this in this study.

5.5. Research Interpretation

The equation for the best surrogate marker response of any sort, including those determined retrospectively, is that of the 16 week TAUC, which has a specificity of 69.63%:

$$\beta = -2.2930 - 0.0866*(\text{CD4\%TAUC16}) + 0.07306\ (\text{Past Major OI}) - 0.3313*(\text{Present RTI use})$$

where

$CD4\%TAUC_{16}$ = time-averaged CD4% change per week over the first 16 weeks

past major OI:	0 = no past OI	1 = past OI
Present RTI use:	0 = no present RTI use	1 = present RTI use

The coefficient for the CD4% $TAUC_{16}$ is negative, implying that CD4% increases are associated with an decreased odds ratio for illness (Table 16, page 57).

Why might the CD4% $TAUC_{16}$ be the best surrogate marker in terms of specificity? The CD4% has been considered a more stable surrogate marker than the CD4, as the latter (and all lymphocytes) may be increased by exercise (but this CD4 increase is transient and of unknown clinical significance), and reduced by viral or parasitic infections. As all lymphocytes are so affected, the CD4% should remain relatively invariable during these perturbations. As such, the CD4% TAUC16 may more reliably indicate the status of the immune system.

Why is the 40 week TAUC not as strongly associated with the endpoint? Had not the surrogate marker responses been censored at the time of opportunistic illness, one could surmise that the predictive value of the $TAUC_{40}$ is "diluted" as compared to the $TAUC_{16}$. Persons who suffered an opportunistic illness received open label ritonavir thereafter (or after 16 weeks if it occurred before this time), which would have increased the $TAUC_{40}$. As such, even those who had an opportunistic illness might have experience good CD4 or CD4% responses; this could therefore reduce the specificity of these surrogate

markers. This potential problem was avoided through censoring; however, there is no obvious explanation otherwise for the reduced specificity of the $TAUC_{40}$ as compared to the $TAUC_{16}$.

Nonetheless, the $TAUC_{40}$ of the CD4, CD4% and CD8 approach 60%, and are superior to early surrogate marker changes after treatment initiation and peak response, suggesting the potential for this surrogate marker response as a correlate of clinical outcome. It is unfortunate that the viral load could not be better assessed because of the small sample size, as its association with illness has been demonstrated in other studies and it is in widespread clinical use. As well, the standard of care is to suppress HIV to below the detection limit of the assay; the association between this degree of viral load change and the development of OIs has not been reported in the literature. However, very few of the subjects in this study achieved suppression of viral load to this degree.

As with the clinical surrogate marker changes of greatest specificity, we determined the positive and negative predictive values, and likelihood ratios, for selected changes in the surrogate marker. These perform in a similar manner to the Week 8 CD4 cell count, so will not be reiterated here.

5.6. Further Statistical Considerations

Generalizability

There are potential hazards in the generalizability of these results to other patient populations. The data presented here is potentially biased, both in statistical manipulation and selection bias. In terms of the former, the corrections made for missing data may have altered the relationship between the surrogate marker response and clinical outcome. In the latter case, four subjects were excluded from this thesis, as no baseline data was present. More importantly, the subjects in the original study were a select group, as 36% of screened subjects did not enter study. It is likely that the study population was healthier than expected, as a large number were excluded because of lab abnormalities or illness. In addition, subjects were required to have had greater than nine months of previous antiretroviral

therapy, which would select for tolerance to therapy.

As with many studies, subjects had closer follow-up and more frequent surrogate marker measures than usual, which may have led to greater compliance than seen in non-study patients. Other biases may have been introduced through provision of open label ritonavir once an opportunistic illness occurred: this may have led to discontinuation of prophylactic medications, especially among those who feel they are receiving placebo (as manifested by unchanging CD4).

Finally, there are concerns that the treatment strategy in M94-247 is outdated. The addition of a single drug to ongoing antiretroviral therapy is no longer the standard of care, and the use of full dose ritonavir alone (as opposed to lower doses in combination with other protease inhibitors) is rarely done because of intolerance.

Nonetheless, except for the potential statistical problems introduced, the associations found may be relatively generalizable to other populations. Most importantly, there is no evidence that surrogate marker changes related to differing antiretroviral treatment regimens are at all different qualitatively. This suggests that the surrogate marker changes assessed here are similar in health benefit (for similar magnitudes and durations of change) to those seen with more modern therapies in less select populations.

5.7. Future Directions

This study has highlighted several areas of potential further study:

1. There is good evidence that the TAUC is a good measure of drug effect, in terms of association with clinical endpoint. This should be assessed in other cohorts of different drugs and combinations, at different stages of HIV infection. Databases with adequate numbers of subjects having viral load measures performed should be assessed.

2. The TAUC should be compared to what has become the standard of care in surrogate marker response, which is the achievement of an "undetectable" viral load.

3. These analyses should also be assessed by other statistical methods, in particular the Cox Proportional Hazards Model and Kaplan Meier method. Consideration should be made of categorization of surrogate marker responses into groups, perhaps quartiles, for assessment.

4. More important than amount of surrogate marker change might be the levels to which they change. For the CD4 cell count, for instance, analysis of the importance of increase *to* 50 cells/μL could be compared to the association of a change *by* 50 cells/μL with the development of clinical endpoint.

References

1. Gottlieb MS, Schroff R, Schanker HM, Weisman JD, Fan PT, Wolf RA, Saxon A. *Pneumocystis carinii* pneumonia and mucosa candidiasis in previously healthy homosexual men: evidence of a new acquired cellular immunodeficiency. N Engl J Med 1981; 305(24):1425-31.

2. Anonymous. Acquired immunodeficiency syndrome (AIDS) among blacks and Hispanics: United States. MMWR 1988; 35(42):655-8.

3. CDC. Mortality attributable to HIV infection/AIDS - United States, 1981-1990. MMWR 1991;40(3):41-4.

4. CDC. Update: Mortality attributable to HIV infection among persons aged 25-44 years - United States, 1994. MMWR 1996;45(6).

5. AIDS in Canada: Quarterly Surveillance Update. Division of HIV/AIDS Surveillance, Bureau of HIV/AIDS and STD, Laboratory Centre for Disease Control. June 30, 1997.

6. Rutherford GW, Lifson AR, Hessol NA, O'Malley PM, Buchbinder SP, Barnhart JL et al. Course of HIV-I infection in a cohort of homosexual and bisexual men: an 11 year follow up study. BMJ 1990; 301(6762):1183-1188.

7. Hoover DR, Saah AJ, Bacellar H, Phair J, Detels R, Anderson R, Kaslow RA. Clinical manifestations of AIDS in the era of *Pneumocystis* prophylaxis. N Engl J Med 1993;329(26):1922-1926.

8. Kravcik S, Hawley-Foss N, Fillion D, Fyke K, Pagé S, Denommé N et al. The causes of death of HIV-infected persons in Ottawa, 1984-1995. Arch Intern Med 1997;157:2069-73.

9. Palella FJ, Delaney KM, Moorman AC, Loveless MO, Fuhrer J, Satten GA et al. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. N Engl J Med 1998;338(13):853-60.

10. Perelson AS, Neumann AU, Markowitz M, Leonard JM, Ho DD. HIV-1 dynamics in vivo: virion clearance rate, infected cell lifespan, and viral generation time. Science 1996;271:1582-86.

11. Raboud J, Montaner JS, Conway B et al. Variation in plasma RNA levels, CD4 cell counts and p24 antigen levels in clinically stable men with human immunodeficiency virus infection. J Infect Dis 1996;174:191-4.

12. Hughes MD, Johnson VA, Hirsch MS, Bremer JW, Elbeik T, Erice A et al. Monitoring plasma HIV-1 RNA levels in addition to CD4+ lymphocyte count improves assessment of antiretroviral therapeutic response. Ann Intern Med 1997;126(12):929-38.

13. Fischl MA, Richmann DD, Grieco MH et al. The efficacy of azidothymidine (AZT) in the treatment of patients with AIDS and AIDS-related complex. A double-blind, placebo-controlled study. N Engl J Med 1987;317:185-91.

14. Fischl MA, Parker CB, Pettielli C et al. A randomized controlled trial of zidovudine in patients with the acquired immunodeficiency syndrome. N Engl J Med 1990;323:1010-25.

15. Hammer SM, Katzenstein DA, Hughes MD et al. A trial comparing nucleoside monotherapy

with combination therapy in HIV-infected adults with CD4 cell counts from 200 to 500 per cubic millimeter. N Engl J Med 1996;335:1081-90.

16. D'Aquila RT, Hughes MD, Johnson VA et al. A randomized, double-blind placebo-controlled trial of nevirapine, zidovudine and didanosine in patients with human immunodeficiency virus type 1 infection. Ann Intern Med 1996;124:1019-31.

17. Montaner JS, Reiss P, Cooper D, Vella S, Harris M, Conway B et al. A randomized, double-blind trial comparing combinations of nevirapine, didanosine and zidovudine for HIV-infected patients: the INCAS Trial. JAMA 1998;279(12):930-7.

18. Hammer SM, Squires KE, Hughes MD, Grimes JM, Demeter LM, Currier JS et al. A controlled trial of two nucleoside analogues plus indinavir in persons with human immunodeficiency virus infection and CD4 cell counts of 200 per cubic millimeter or less. (ACTG 320) N Engl J Med 1997;337(11):725-33, 1997.

19. Masur H et al. CD4 counts as predictors of opportunistic pneumonias in human immunodeficiency virus (HIV) infection. Ann Intern Med 1989;111:223.

20. Rachlis AR, Zarowny DP and the Canadian HIV Trials Network Antiretroviral Working Group. Guidelines for antiretroviral therapy for HIV infection. Can Med Assoc J 1998;158(4):496-505.

21. Panel on Clinical Practices for Treatment of HIV Infection/Department of Health and Human Services. Guidelines for the use of antiretroviral agents in HIV-infected adults and adolescents.

22. Cameron DW, Heath-Chiozzi M, Danner S, Cohen C, Kravcik S, Maurath C et al. Randomised, placebo-controlled trial of ritonavir in advanced HIV-1 disease. Lancet 1998; 351(9102):543-9.

23. Gelman R, Cheng S, Kidd P, Waxdal M, Kagan J. Assessment of the effects of instrumentation, monoclonal antibody, and fluorochrome on flow cytometric immunophenotyping: a report based on 2 years of the NIAID DAIDS Flow Cytometry Quality Assessment Program. Clin Immunol Immunopathol 1993;66:150-62.

24. Hughes MD, Stein DS, Gundacker HM, Valentine FT, Phair JP, Volberding PA. Within-subject variation in CD4 lymphocyte count in asymptomatic human immunodeficiency virus infection: implications for patient monitoring. J Infect Dis 1994;169(1):28-36.

25. Phillips AN, Lee CA, Elfords J, Janossy G, Timms A, Bofill M et al. Serial CD4 lymphocyte counts and development of AIDS. Lancet 1991;337:389-92.

26. Eyster ME, Gail MH, Ballard JO, Al-Mondhiry H, Goegert JJ. Natural history of human immunodeficiency virus infection in hemophiliacs: effects of T cell subsets, platelet counts and age. Ann Intern Med 1987;107:1-6.

27. Lange JMA, de Wolfe F, Goudsmit J. Markers for progression in HIV infection. AIDS 1989;3(suppl 1):153-60.

28. Schellekens PTA, Tersmette M, Roos MTL et al. Biphasic rate of CD4+ cell count decline during progression to AIDS correlates with HIV-1 phenotype. AIDS 1992;6:665-9

29. Polk BF, Fox R, Brookmeyer R, Kanchanaraksa S, Kaslow R, Visscher B, Rinaldo C, Phair

J. Predictors of the acquired immunodeficiency syndrome developing in a cohort of seropositive homosexual men. N Engl J Med 1987;316(2):61-6.

30. Hughes MD, Johnson VA, Hirsch MS, Bremer JW, Elbeik T, Erice A et al. Monitoring plasma HIV-1 RNA levels in addition to CD4+ lymphocyte count improves assessment of antiretroviral therapeutic response. Ann Intern Med 1997;126(12):929-38.

31. Mellors JW, Rinaldo CR Jr, Gupta P, White RM, Todd JA, Kingsley LA. Prognosis in HIV-1 infection predicted by the quantity of virus in plasma. Science 1996;272:1167-70.

32. Spijkerman IJB, Prins M, Goudsmit J, Veugelers PJ, Coutinho RA, Miedema F, de Wolf F. Early and late HIV-1 RNA level and its association with other markers and disease progression in long term AIDS-free homosexual men. J Acquir Immune Defic Syndr 1997;11(11):1383-88.

33. Deeks SG, Coleman RL, White R, Pachl C, Schambelan M, Chernoff DN, Feinberg MB. Variance of plasma human immunodeficiency virus type 1 RNA levels measured by branched DNA within and between days. J Infect Dis 1997;176(2):514-7.

34. Donovan RM, Bush CE, Markowitz NP, Baxa DM, Saravolatz LD. Changes in virus load markers during AIDS-associated opportunistic disease in human immunodeficiency virus-infected persons. J Infect Dis 1996;174(2):401-3.

35. Bush CE, Donovan RM, Markowitz NP, Kvale P, Saravolatz LD. A study of HIV RNA viral load in AIDS patients with bacterial pneumonia. J Acquir Immune Defic Syndr Hum Retrovir 1996;13(1):23-6.

36. Fahey JL, Taylor JM, Detels R, Hofmann B, Melmed R, Nishanian P et al. The prognostic value of cellular and serologic markers in infection with human immunodeficiency virus type 1. N Engl J Med 1990;322(3):166-772.

37. Saah AJ, Munoz A, Kuo V, Fox R, Kaslow RA, Phair JP *et al.* Predictors of the risk of development of acquired immunodeficiency syndrome within 24 months among gay men seropositive for human immunodeficiency virus type 1: a report from the Multicenter AIDS Cohort Study. Am J Epidemiol 1992;135(10):1147-55.

38. Page JB et al. Predictors of Survival in Human Immunodeficiency Virus Type 1-Seropositive Intravenous Drug Users. Clinical and Diagnostic Laboratory Immunology 1996;51-60.

39. Mellors JW, Kingsley LA, Rinaldo CR, Todd JA, Hoo BS, Kokka RP, Gupta P. Quantitation of HIV-1 RNA in plasma predicts outcome after seroconversion. Ann Intern Med 1995;122(8):573-9.

40. Mellors JW, Muñoz A, Giorgi JV, Margolick JB, Tassoni CJ, Gupta P et al. Plasma viral load and CD4+ lymphocytes as prognostic markers of HIV-1 infection. Ann Intern Med 1997;126(12): 946-54.

41. O'Brien TR, Blattner WA, Waters D, Eyster E, Hilgartner MW et al Serum HIV-1 RNA levels and time to development of AIDS in the Multicenter Hemophilia Cohort Study. JAMA 1996;276(2):105-10.

42. Craib KJ, Strathdee SA, Hogg RS, Leung B, Montaner JS, O'Shaughnessy MV, Schechter MT. Serum levels of human immunodeficiency virus type 1 (HIV-1) RNA after seroconversion: a predictor of long-term mortality in HIV infection. J Infect Dis

1997;176(3):798-800.

43. Vlahov D, Graham N, Hoover D, Flynn C, Bartlett JG, Margolick JB et al. Prognostic indicators for AIDS and infectious disease death in HIV-infected injection drug users: plasma viral load and CD4+ cell count. JAMA 1998;279(1):35-40.

44. Galetto-Lacour A, Yerly S, Perneger TV, Baumberger C, Hirschel B, Perrin L. Prognostic value of viremia in patients with long-standing human immunodeficiency virus infection. Swiss HIV Cohort Study Group. J Infect Dis 1996;173(6):1388-93.

45. Pedersen C, Katzenstein T, Nielsen C, Lundgren JD, Gerstoft J. Prognostic value of serum HIV RNA levels at virologic steady state after seroconversion: relation to CD4 cell count and clinical course of primary infection. J Acquir Immune Defic Syndr Hum Retrovir 1997;16(2):93-9.

46. Cotte L, Trabaud MA, Rougier P, Bailly F, Chapuis F, Trepo C. Predictive value of HIV-1 RNA detection in plasma by branched DNA assay during long-term zidovudine therapy. Eur J Clin Microbiol Infect Dis 1996;15(8):639-45.

47. Pantaleo G, Menzo S, Vaccarezza M, Graziosi C, Cohen OJ, Demarest JF et al. Studies in subjects with long-term nonprogressive human immunodeficiency virus infection. New Engl J Med 1995;332(4):209-216.

48. Cao Y, Qin L, Zhang L, Safrit J, Ho DD. Virologic and immunologic characterization of long-term survivors of human immunodeficiency virus type 1 infection. N Engl J Med 1995;332(4):201-208.

49. Farzadegan H, Henrard DR, Kleeberger CA, Schrager L, Kirby AJ, Saah AJ et al. Virologic and serologic markers of rapid progression to AIDS after HIV-1 seroconversion. J Acquir Immune Def Human Retrovirol 1996;13(5):448-55.

50. Shearer WT, Quinn TC, LaRussa P, Lew JF, Mofenson L, Almy S et al. Viral Load and disease progression in infants infected with human immunodeficiency virus type 1. N Engl J Med 1997;336(19):1337-42.

51. Mofenson LM, Korelitz J, Meyer WA, Betheel J, Rich K, Pahwa S et al. The relationship between serum human immunodeficiency virus type 1 RNA level, CD4 lymphocyte percent, and long-term mortality risk in HIV-1-infected children. J Infect Dis 1997;175(5):1029-38.

52. Choi S, Lagakos SW, Schooley RT, Volberding PA. CD4+ lymphocytes are an incomplete surrogate marker for clinical progression in persons with asymptomatic HIV infection taking zidovudine. Ann Intern Med 1993;118(9):674-80.

53. Jacobson MA, De Gruttola V, Reddy M, Arduino JM, Strickland S, Reichman RC et al. The predictive value of changes in serologic and cell markers of HIV activity for subsequent clinical outcome in patients with asymptomatic HIV disease treated with zidovudine. AIDS 1995;9:727-34.

54. Graham NM, Piantadosi S, Park LP, Phair JP, Rinaldo CR, Fahey JL. CD4+ lymphocyte response to zidovudine as a predictor of AIDS-free time and survival time. J Acquir Immune Defic Syndr 1993;6(11):1258-66.

55. Graham NMH, Park LP, Piantadosi S, Phair JP, Mellors J, Fahey JL, Saah AJ. Prognostic value of combined response markers among human immunodeficiency virus-infected persons:

possible aid in the decision to change zidovudine monotherapy. Clin Infect Dis 1995;20:352-62.

56. Goldman AI, Carlin BP, Crane LR, Launer C, Korvick JA, Deyton L, Abrams DI. Response of CD4 lymphocytes and clinical consequences of treatment using ddI or ddC in patients with advanced HIV infection. J Acquir Immune Def Human Retrovirol 1996;11(2):161-9.

57. Drusano GL, Stein DS. Mathematical modelling of the interrelationship of CD4 lymphocyte count and viral load changes induced by the protease inhibitor indinavir. Antimicrob Agents Chemother 1998;42(2):358-61.

58. Marschner IC, Collier AC, Coombs RW, D'Aquila RT, DeGrutola V, Fischl MA et al. Use of changes in plasma levels of human immunodeficiency virus type 1 RNA to assess the clinical benefit of antiretroviral therapy. J Infect Dis 1998;177:40-7.

59. O'Brien WA, Hartigan PM, Martin D, Esinhart J, Hill A, Benoit S et al. Changes in plasma HIV-1 RNA and CD4+ lymphocyte counts and the risk of progression to AIDS. N Engl J Med 1996;334(7):426-431.

60. O'Brien WA, Hartigan PM, Daar ES, Simberkoff MS, Hamilton JD et al. Changes in plasma HIV RNA levels and CD4+lymphocyte counts predict both response to antiretroviral therapy and therapeutic failure. Ann Intern Med 1997;126(12):939-45.

61. Coombs RW, Welles SL, Hooper C, Reichelderfer PS, D'Aquila RT, Japour AJ et al. Association of plasma human immunodeficiency virus type 1 RNA level with risk of clinical progression in patients with advanced infection. J Infect Dis 1996;174(4):704-12.

62. Welles SL, Jackson JB, Yen-Lieberman B, Demeter L, Japour AJ, Smeaton LM et al. Prognostic value of plasma human immunodeficiency virus type 1 RNA levels in patients with advanced HIV-1 disease and with little or no prior zidovudine therapy. J Infect Dis 1996;174(4):696-703.

63. Katzenstein DA, Hammer SM, Hughes MD, Gundacker H, Jackson JB, Fiscus S et al. The relation of virologic and immunologic markers to clinical outcomes after nucleoside therapy in HIV-infected adults with 200 to 500 CD4 cells per cubic millimetre. N Engl J Med 1996;335(15):1091-8.

64. Fiscus SA, Hughes MD, Lathey JL, Pi T, Jackson JB, Rasheed S et al. Changes in virologic markers as predictors of CD4 cell decline and progression of disease in human immunodeficiency virus type 1-infected adults treated with nucleosides. J Infect Dis 1998;177:625-33.

65. Lathey JL, Hughes MD, Fiscus SA, Pi T, Jackson JB, Rasheed S et al. Variability and prognostic values of virologic and CD4 cell measures in human immunodeficiency virus type 1-infected patients with 200-500 CD4 cells/mm. J Infect Dis 1996;177(3):617-24.

66. Phillips AN, Eron JJ, Barlett JA, Rubin M, Johnson J, Price S, Hill AM. HIV-1 RNA levels and the development of clinical disease. AIDS 1996;10(8):859-65.

67. Montaner JS, DeMasi R, Hill AM. The effects of lamivudine treatment on HIV-1 disease progression are highly correlated with plasma HIV-1 RNA and CD4 cell count. AIDS 1998;12(5):F23-8.

68. Åsjö B, Morfeldt-Månson L, Albert J, Biberfeld G, Karlsson A, Lidman K, Fenyö EM.

Replicative capacity of human immunodeficiency virus from patients with varying severity of HIV infection. Lancet 1986;2:660-2.

69. Tersmette M, Gruters RA, de Wolf F, de Goede REY, Lange JMA, Schellekens PTA et al. Evidence for a role of virulent human immunodeficiency virus (HIV) variants in the pathogenesis of acquired immunodeficiency syndrome: studies on sequential HIV isolates. J Virol 1989;63:2118-25.

70. Tersmette M, Lange JMA, de Goede REY, de Wolf F, Eeftinck Schattenkerk JKM, Schellekens PTA et al. Association between biologic properties of human immunodeficiency virus variants and risk for AIDS and AIDS mortality. Lancet 1989;1:983-5.

71. Phair J, Munoz A, Detels R et al. The risk of *Pneumocystis carinii* pneumonia among men infected with human immunodeficiency virus type 1. N Engl J Med 1990;322:161-5.

72. Klein RS, Harris CA, Small CB, Moll B, Lesser M, Friedland GH. Oral candidiasis in high risk patients as the initial manifestation of the acquired immunodeficiency syndrome. N Engl J Med 1984;311:354-8.

73. Greenspan D, Greenspan DS, Hearst NG et al. Relation of oral hairy leukoplakia to infection with human immunodeficiency virus and the risk of developing AIDS. J Infect Dis 1987;155:475-81.

74. Kaplan JE, Spira TJ, Fishbein DB et al. A six year follow up of HIV-infected homosexual men with lymphadenopathy. Evidence for an increased risk for developing AIDS after the third year of lymphadenopathy. JAMA 1988;260:2694-7.

75. Melbye M, Goedert JJ, Grossman JR, Eyster ME, Biggar RE. Risk of AIDS after herpes zoster. Lancet 1987;1:728-30.

76. The Delta Coordinating Committee. Delta: a randomized double-blind controlled trial comparing combinations of zidovudine plus didanosine or zalcitabine with zidovudine alone in HIV-infected individuals. Lancet 1996;348:283-91.

77. Gulick R, Mellors J, Havlir D, Eron J, Gonzalez C, McMahon D et al. Potent and Sustained Antiretroviral Activity of Indinavir (IDV) in Combination with Zidovudine (ZDV) and Lamivudine (3TC) [Abstract]. Third Conference on Retroviruses and Opportunistic Infections 1996.

78. Munoz A, Bass S, Saah A, Chmiel J, Taylor J, Kingsley L. AIDS-free time after HIV-1 seroconversion in homosexual men according to demographic subgroups [Abstract W.A.P.68]. Int Conf AIDS 1989; 5:131.

79. Saravolatz LD, Winslow DL, Collins G, Hodges JS, Pettinelli C, Stein DS et al. Zidovudine alone or in combination with didanosine or zalcitabine in HIV-infected patients with the acquired immunodeficiency syndrome or fewer than 200 CD4 cells per cubic millimeter. New England Journal of Medicine 1996; 335(15):1099-106.

80. Cameron DW, Japour AJ, Xu Y, Hsu A, Mellors J, Farthing C et al. Ritonavir and saquinavir combination therapy for the treatment of HIV infection. AIDS 1999;13(2):213-224.

81. Richman DD, Fischl MA, Grieco MH, Gottlieb MS, Volberding PA, Laskin et al. The toxicity of azidothymidine (AZT) in the treatment of patients with AIDS and AIDS-related complex. A double-blind, placebo-controlled trial. N Engl J Med 1987; 317(4):192-7.

82. Kempf DJ, Rode RA, Xu Y, Sun E, Heath-Chiozzi ME, Valdes J et al. The duration of viral suppression during protease inhibitor therapy for HIV-1 infection is predicted by plasma HIV-1 at the nadir. AIDS 1998;12(5):F9-14.

Appendix 1

1993 Centers for Disease Control Classification of AIDS-Defining Illnesses

Infectious

Candidiasis, esophageal, tracheal, etc

Cervical Carcinoma

CMV Retinitis and other CMV infections

Coccidioidomycosis, extrapulmonary

Cryptococcal Meningitis

Cryptosporidiosis, chronic intestinal

Herpes simplex: chronic ulcer, esophagitis, pneumonitis, etc

Histoplasmosis, disseminated or extrapulmonary

Isosporiasis, chronic intestinal

Mycobacterium avium complex or other atypical mycobacterial infection

Pneumocystis carinii pneumonia

Progressive Multifocal Leukoencephalopathy

Recurrent Bacterial Pneumonia

Salmonella bacteremia, recurrent

Toxoplasmosis, Cerebral

Tuberculosis

Non-Infectious

AIDS Wasting Syndrome

HIV Encephalopathy/AIDS Dementia Complex

Kaposi's Sarcoma

Lymphoma

(MMWR 1992;41:RR-17)

Definitions for Appendices 2-8

n = sample size

S.D. = standard deviation

IQR = interquartile range

-2LL = -2(log-likelihood)

Hlχ2 = Hosmer-Lemeshow chi square

sig = level of significance

Modelχ^2 = model chi square

Wald = Wald statistic

%correct = % correctly classified by logistic regression equation

spec = specificity of logistic regression equation

sens = sensitivity of logistic regression equation

β = coefficient of surrogate marker response

OR = odds ratio of surrogate marker response

Appendix 2.

Description of Covariates

CD4 (all subjects)

	n	Range	Mean	S.D.	Median	IQR	Normality
Baseline	1052	0, 155.00	31.60	28.28	20.00	10.00, 44.00	no
Week 2	951	-48.00, 533.00	15.17	43.19	2.00	-2.00, 19.00	no
Week 4	944	-66.00, 542.00	23.52	58.22	3.00	-2.00, 26.00	no
Week 8	913	-74.00, 403.00	23.67	56.48	3.00	-0.50, 2.00	no
Week 12	860	-95.00, 338.00	21.11	52.14	3.00	-0.50, 2.00	yes
Week 16	818	-74.00, 421.00	22.03	53.26	4.00	-0.38, 2.50	no
Peak	1035	-45.00, 542.00	40.96	70.49	13.00	2.00, 52.00	no
$TAUC_{10}$	1008	-64.56, 293.81	18.05	41.34	2.63	-0.94, 20.57	no
$TAUC_{40}$	1010	-63.57, 244.68	17.68	40.42	2.70	-0.84, 22.51	no

CD4% (all subjects)

	n	Range	Mean	S.D.	Median	IQR	Normality
Baseline	1011	0, 24.00	3.92	3.47	3.00	1.50, 5.50	no
Week 2	917	-7.00, 27.00	1.35	3.46	0.50	-0.50, 2.00	no
Week 4	910	-6.50, 33.00	1.31	3.39	0.50	-0.50, 2.00	no
Week 8	885	+14.00, 14.00	0.96	2.67	0.50	-2.00, 30.50	no
Week 12	833	-11.00, 22.00	1.13	2.97	0.50	-3.00, 25.00	yes
Week 16	792	-12.00, 23.00	1.28	3.04	0.50	-3.00, 27.25	no
Peak	995	-6.00, 33.00	2.82	4.20	1.50	0.50, 4.00	no
$TAUC_{10}$	968	-15.00, 45.00	1.23	3.16	0.44	-0.19, 1.75	no
$TAUC_{40}$	968	-7.75, 16.97	1.03	2.33	0.43	-0.13, 1.53	no

CD8 (all subjects)

	n	Range	Mean	S.D.	Median	IQR	Normality
Baseline	1024	0.30, 3604.50	480.87	328.87	409.25	243.13, 657.25	no
Week 2	913	-1092.50, 1244.50	21.75	198.64	4.50	-86.25, 106.50	no
Week 4	908	-939.00, 3036.50	108.02	376.33	17.00	-73.38, 169.38	no
Week 8	880	-1134.00, 2767.00	150.72	435.56	21.25	-78.63, 252.88	no
Week 12	829	-1372.00, 3580.50	106.00	391.98	23.00	-98.50, 210.25	yes
Week 16	784	-1346.00, 2292.50	93.76	349.64	23.50	-86.50, 215.50	no
Peak	996	-1092.50, 3580.50	297.47	464.54	150.75	23.50, 405.25	no
$TAUC_{16}$	976	-2032.63, 4321.50	100.14	370.89	18.51	-57.61, 160.63	no
$TAUC_{40}$	976	-1094.85, 1729.99	80.43	253.93	22.73	-48.80, 163.65	no

CD8% (all subjects)

	n	Range	Mean	S.D.	Median	IQR	Normality
Baseline	1011	5.00, 88.00	57.51	15.30	60.00	48.00, 69.00	yes
Week 2	917	-60.00, 28.00	-1.59	6.59	-1.00	-5.00, 2.00	yes
Week 4	910	-36.00, 28.00	-0.22	7.56	-0.50	-5.00, 4.00	yes
Week 8	885	-66.00, 36.50	0.86	9.27	0.50	-4.50, 6.00	yes
Week 12	833	-39.00, 32.00	0.52	9.08	0.00	-4.50, 6.00	yes
Week 16	792	-48.00, 44.50	0.09	9.48	0.00	-5.00, 5.00	yes
Peak	995	-48.00 , 44.50	5.51	7.93	4.50	0.50, 9.50	yes
$TAUC_{16}$	975	-88.00, 90.00	0.43	9.48	0.00	-3.75, 4.06	yes
$TAUC_{40}$	977	-26.00, 63.50	0.06	6.56	-0.13	-3.52, 3.38	no

Viral Load (all subjects)

	n	Range	Mean	S.D.	Median	IQR	Normality
Baseline	171	0.70, 6.33	5.24	0.59	5.28	4.92, 5.57	Not done
Week 2	149	-2.88, 0.90	-0.58	0.88	-0.12	-1.39, 0.11	Not done
Week 4	147	-3.22, 0.69	-0.52	0.94	-0.08	-1.06, 0.11	Not done
Week 8	143	-3.76, 0.86	-0.46	0.97	-0.13	-0.53, 0.16	Not done
Week 12	136	-3.76, 0.96	-0.34	0.79	-0.11	-0.61, 0.12	Not done
Week 16	135	-3.76, 0.93	-0.28	0.78	-0.09	-0.53, 0.15	Not done
Peak	156	-3.76, 0.58	-0.95	1.00	-0.63	-1.74, -0.11	Not done
$TAUC_{10}$	147	-8.20, 1.76	-0.40	0.97	-0.16	-0.59, 0.04	Not done
$TAUC_{10}$	147	-3.45, 0.53	-0.36	0.65	-0.16	-0.49, 0.01	Not done

Appendix 3.

Unadjusted Univariate Logistic Regression: All Subjects

CD4 (all subjects, no baseline variables)

	n	-2LL	HLχ^2	sig	Modelχ^2	sig	Wald	sig	%correct	spec	sens	β	OR	95% CI
Baseline	1052	1113.70	9.29	0.23	23.44	0.00	20.14	0.00	50.29	45.49	66.26	-0.01	0.9859	0.9798, 0.9920
peak	1035	1090.36	9.99	0.27	35.41	0.00	24.06	0.00	50.92	41.74	80.99	-0.01	0.9912	0.9877, 0.9947
$TAUC_{16}$	1008	1023.95	28.78	0.00	33.83	0.00	23.13	0.00	55.06	50.25	72.27	-0.02	0.9849	0.9788, 0.9910
$TAUC_{40}$	1010	1019.20	41.43	0.00	39.56	0.00	27.05	0.00	56.93	52.28	73.64	-0.02	0.9388	0.9771, 0.9895

CD4% (all subjects, no baseline variables)

	n	-2LL	HLχ^2	sig	Modelχ^2	sig	Wald	sig	%correct	spec	sens	β	OR	95% CI
Baseline	1011	1076.91	4.27	0.83	9.79	0.00	8.88	0.00	54.50	54.49	54.55	-0.07	0.9290	0.8851, 0.9751
peak	995	1065.92	14.49	0.07	10.02	0.00	8.53	0.00	52.76	49.80	62.61	-0.07	0.9368	0.8966, 0.9787
$TAUC_{16}$	968	1002.18	20.06	0.01	0.00	1.00	0.00	1.00	78.72	100.00	0.00	0.00	1.0000	0.9524, 1.0499
$TAUC_{40}$	968	984.32	23.86	0.00	17.86	0.00	15.45	0.00	65.70	76.51	25.73	-0.16	0.8495	0.7831, 0.9215

CD8 (all subjects, no baseline variables)

	n	-2LL	HLχ^2	sig	Modelχ^2	sig	Wald	sig	%correct	spec	sens	β	OR	95% CI
Baseline	1024	1095.34	6.00	0.65	5.41	0.02	5.03	0.02	56.93	60.00	46.58	0.00	0.9994	0.9989, 0.9999
peak	996	1056.77	14.36	0.07	17.28	0.00	14.22	0.00	52.71	48.76	65.94	0.00	0.9992	0.9988, 0.9996
$TAUC_{16}$	976	1005.25	26.65	0.00	3.37	0.07	3.04	0.08	79.10	97.66	10.14	0.00	0.9996	0.9991, 1.0001
$TAUC_{40}$	976	980.20	21.04	0.01	28.42	0.00	23.71	0.00	64.45	72.56	34.30	0.00	0.9981	0.9973, 0.9988

CD8% (all subjects, no baseline variables)

	n	-2LL	HLχ^2	sig	Modelχ^2	sig	Wald	sig	%correct	spec	sens	β	OR	95% CI
Baseline	1011	1085.90	1.76	0.99	0.81	0.37	0.82	0.37	68.55	83.72	17.32	0.00	0.9956	0.9862, 1.0051
peak	995	1075.77	3.66	0.89	0.16	0.69	0.16	0.69	75.08	96.73	3.04	0.00	0.9962	0.9778, 1.0150
$TAUC_{16}$	975	1008.75	29.78	0.00	2.03	0.15	2.04	0.15	78.97	97.65	10.10	0.01	1.0117	0.9957, 1.0281
$TAUC_{40}$	977	1016.71	14.03	0.08	0.22	0.64	0.22	0.64	78.61	100.00	0.48	0.01	1.0055	0.9826, 1.0289

Viral Load (all subjects, no baseline variables)

	n	-2LL	HLχ^2	sig	Modelχ^2	sig	Wald	sig	%correct	spec	sens	β	OR	95% CI
Baseline	171	192.22	9.25	0.32	0.64	0.42	0.60	0.44	37.43	28.13	65.12	0.26	1.2945	0.6723, 2.4926
peak	156	176.86	7.52	0.48	2.85	0.09	2.65	0.10	48.08	37.39	78.05	0.33	1.3846	0.9357, 2.0489
$TAUC_{16}$	147	158.93	7.88	0.45	0.08	0.78	0.07	0.78	80.27	100.00	14.71	0.05	1.0612	0.6928, 1.6256
$TAUC_{40}$	147	153.14	11.16	0.19	5.87	0.02	4.19	0.04	57.14	51.33	76.47	0.99	2.7099	1.0435, 6.9903

Appendix 4

Adjusted Univariate Logistic Regression: All Subjects

CD4 (all subjects, with adjustment for baseline variables)

	n	-2LL	HLχ^2	sig	Modelχ^2	sig	Wald	sig	%correct	spec	sens	β	OR	95% CI
Baseline	966	1040.89	4.32	0.83	19.54	0.00	12.68	0.00	49.90	45.11	65.22	-0.01	0.9880	0.9815, 0.9946
peak	950	1014.83	1.69	0.99	34.53	0.00	20.43	0.00	52.21	45.77	72.49	-0.01	0.9919	0.9884, 0.9954
$TAUC_{16}$	923	947.12	22.40	0.00	35.43	0.00	20.19	0.00	54.93	51.26	67.63	-0.01	0.9855	0.9793, 0.9918
$TAUC_{40}$	925	941.81	25.05	0.00	41.75	0.00	24.29	0.00	54.16	50.00	68.60	-0.02	0.9834	0.9769, 0.9900

CD4% (all subjects, with adjustment for baseline variables)

	n	-2LL	HLχ^2	sig	Modelχ^2	sig	Wald	sig	%correct	spec	sens	β	OR	95% CI
Baseline	929	1004.36	2.25	0.97	12.67	0.01	4.38	0.04	51.67	47.39	65.45	-0.05	0.9469	0.8998, 0.9965
peak	914	989.54	4.83	0.78	17.00	0.00	7.56	0.01	52.52	48.20	66.21	-0.06	0.9393	0.8982, 0.9822
$TAUC_{16}$	887	926.23	16.00	0.04	8.15	0.04	0.09	0.77	57.73	53.96	73.85	-0.05	0.9537	0.9162, 0.9863
$TAUC_{40}$	887	907.28	16.25	0.04	27.15	0.00	15.40	0.00	57.38	55.78	63.08	-0.18	0.8392	0.7689, 0.9160

CD8 (all subjects, with adjustment for baseline variables)

	n	-2LL	HLχ^2	sig	Modelχ^2	sig	Wald	sig	%correct	spec	sens	β	OR	95% CI
Baseline	942	1021.56	14.55	0.07	9.52	0.02	2.50	0.11	49.04	44.65	63.23	0.00	0.9996	0.9991, 1.0001
peak	915	983.35	14.13	0.08	21.42	0.00	11.27	0.00	53.99	49.64	67.89	0.00	0.9993	0.9989, 0.9997
$TAUC_{16}$	895	931.07	17.72	0.02	9.81	0.02	1.66	0.20	53.97	50.93	64.80	0.00	0.9997	0.9992, 1.0002
$TAUC_{40}$	895	908.34	13.47	0.10	32.54	0.00	20.56	0.00	58.88	58.37	60.71	0.00	0.9982	0.9974, 0.9990

CD8% (all subjects, with adjustment for baseline variables)

	n	-2LL	HLχ^2	sig	Modelχ^2	sig	Wald	sig	%correct	spec	sens	β	OR	95% CI
Baseline	929	1009.00	3.92	0.86	8.00	0.05	0.01	0.92	43.38	33.00	76.82	0.00	0.9995	0.9897, 1.0094
peak	914	998.11	3.44	0.90	8.44	0.04	0.12	0.73	43.76	33.38	76.71	0.00	0.9966	0.9777, 1.0158
$TAUC_{16}$	894	930.24	28.70	0.00	12.08	0.01	4.32	0.04	56.71	58.82	49.24	0.02	1.0177	1.0010, 1.0347
$TAUC_{40}$	896	939.90	16.84	0.03	9.06	0.03	0.20	0.65	46.88	39.02	74.37	0.01	1.0055	0.9817, 1.0298

Viral Load (all subjects, with adjustment for baseline variables)

	n	-2LL	HLχ^2	sig	Modelχ^2	sig	Wald	sig	%correct	spec	sens	β	OR	95% CI
Baseline	154	178.95	9.50	0.30	1.53	0.68	0.32	0.57	44.16	29.46	83.33	0.18	1.2014	0.6364, 2.2678
peak	140	164.65	4.26	0.83	2.87	0.41	2.13	0.14	45.71	32.00	80.00	0.30	1.3486	0.9027, 2.0147
$TAUC_{16}$	132	146.45	7.14	0.52	2.01	0.57	0.05	0.82	43.18	30.30	81.82	0.05	1.0543	0.6693, 1.6607
$TAUC_{40}$	132	140.67	2.32	0.97	7.79	0.05	4.10	0.04	53.79	45.45	78.79	1.01	2.7375	1.0325, 7.2579

Appendix 5

Unadjusted Univariate Logistic Regression: Ritonavir Subjects

CD4 (ritonavir subjects, no baseline variables)

	n	-2LL	III χ^2	sig	Model χ^2	sig	Wald	sig	%correct	spec	sens	β	OR	95% CI
Baseline	527	451.72	7.12	0.42	10.63	0.00	8.76	0.00	46.87	41.99	72.62	-0.02	0.9719	0.9719, 0.9942
week 2	458	396.32	5.86	0.66	2.15	0.14	1.77	0.18	46.72	41.71	73.61	0.00	0.9897	0.9897, 1.0020
week 4	459	388.58	8.92	0.35	10.23	0.00	7.55	0.01	50.11	45.48	75.00	-0.01	0.9869	0.9869, 0.9978
week 8	453	366.11	10.42	0.24	17.04	0.00	11.80	0.00	57.17	52.99	80.88	-0.01	0.9824	0.9824, 0.9952
week 12	418	336.73	7.30	0.50	14.22	0.00	10.61	0.00	58.23	56.65	67.21	-0.01	0.9828	0.9828, 0.9957
week 16	407	309.41	14.80	0.06	12.96	0.00	9.13	0.00	60.69	59.66	67.27	-0.01	0.9816	0.9816, 0.9960
peak	512	432.88	11.40	0.18	20.91	0.00	14.39	0.00	52.34	47.32	78.31	-0.01	0.9862	0.9862, 0.9956
$TAUC_{16}$	499	411.85	14.56	0.07	18.67	0.00	20.19	0.00	54.93	51.26	67.63	-0.01	0.9855	0.9793, 0.9918
$TAUC_{40}$	500	386.22	9.70	0.29	25.94	0.00	16.88	0.00	57.20	53.97	76.39	-0.02	0.9802	0.9709, 0.9896

CD4% (ritonavir subjects, no baseline variables)

	n	-2LL	HLχ^2	sig	Modelχ^2	sig	Wald	sig	%correct	spec	sens	β	OR	95% CI
Baseline	506	434.22	2.53	0.96	7.53	0.01	6.38	0.01	47.43	43.66	67.56	-0.12	0.8907	0.8143, 0.9744
week 2	441	382.57	16.80	0.03	0.00	0.00	0.03	0.00	84.35	100.00	0.00	0 00	1.0005	0.9398, 1.0650
week 4	441	373.86	8.58	0.38	5.33	0.04	4.32	0.04	52.83	51.21	61.76	-0.09	0.9098	0.8322, 0.9946
week 8	438	366.16	4.89	0.67	5.18	0.03	4.68	0.03	54.57	52.15	68.18	-0.11	0.8947	0.8089, 0.9896
week 12	409	337.80	4.86	0.68	6.76	0.02	5.86	0.02	56.97	56.03	62.30	-0.12	0.8842	0.8003, 0.9768
week 16	395	303.81	9.23	0.32	7.65	0.01	6.58	0.01	68.61	71.64	49.06	-0.14	0.8657	0.7753 , 0.9666
peak	492	430.45	9.40	0.31	3.11	0.10	2.74	0.10	45.73	40.68	72.15	-0.05	0.9511	0.8963, 1.0092
$TAUC_{16}$	478	387.44	8.75	0.36	7.09	0.01	6.49	0.01	76.15	85.64	17.91	-0.08	0.9219	0.8364, 0.9648
$TAUC_{40}$	480	385.61	14.04	0.08	9.64	0.00	8.10	0.00	61.04	61.80	56.52	-0.19	0.8311	0.7316, 0.9440

CD8 (ritonavir subjects, no baseline variables)

	n	-2LL	HLχ^2	sig	Modelχ^2	sig	Wald	sig	%correct	spec	sens	β	OR	95% CI
Baseline	512	441.55	6.84	0.55	2.25	0.13	2.08	0.15	53 32	52.31	58.75	0.00	0.9994	0.9986, 1.0002
week 2	440	378.37	6.78	0.56	0.48	0 49	0.46	0.50	63.41	69.89	27..94	0.00	0.9995	0.9982, 1.0009
week 4	440	377.88	6.92	0.55	0.98	0.32	0.90	0.34	57.73	53.64	67.69	0.00	0.9997	0.9991, 1.0003
week 8	436	355.98	3.01	0.93	11.24	0.00	9.13	0.00	55.73	53.64	67.69	0.00	0.9989	0.9982, 0.9996
week 12	407	337.27	10.21	0.25	6.64	0.01	5.67	0.02	58.23	56.65	67.21	0.00	0.9991	0.9983, 0.9998
week 16	393	302.17	7.31	0.50	4.97	0.03	4.35	0.04	76.59	83.87	28.85	0.00	0.9990	0.9981, 0.9999
peak	494	425.08	3.99	0.86	9.18	0.00	7.55	0.01	50.20	46.02	72.15	0.00	0.9992	0.9986, 0.9998
$TAUC_{16}$	485	393.18	15.78	0 05	0.03	0.87	0.03	0.87	86.19	100 00	1.47	0.00	1.0001	0.9994, 1.0007
$TAUC_{40}$	485	380.04	10.43	0 24	13.14	0.00	10.88	0.00	61.86	62.59	57.35	0.00	0.9980	0.9968, 0.9992

CD8% (ritonavir subjects, no baseline variables)

	n	-2LL	HL χ^2	sig	Model χ^2	sig	Wald	sig	%correct	spec	sens	β	OR	95% CI
Baseline	506	440.46	1.62	0.99	1.29	0.26	1.31	0.25	59.49	62.21	45.00	-0.01	0.9913	0.9767, 1.0062
week 2	441	380.92	4.21	0.84	1.66	0.20	1.64	0.20	55.10	57.53	42.03	0.03	1.0263	0.9863, 1.0679
week 4	441	379.08	6.62	0.58	0.11	0.74	0.11	0.74	73.47	83.91	16.18	0.01	1.0050	0.9755, 1.0353
week 8	438	371.33	6.23	0.42	0.00	0.95	0.00	0.95	84.93	100.00	0.00	0.00	1.0008	0.9756, 1.0267
week 12	409	344.29	15.05	0.06	0.27	0.60	0.27	0.60	78.00	89.66	11.48	-0.01	0.9927	0.9658, 1.0203
week 16	395	309.19	6.71	0.57	2.27	0.13	2.26	0.13	77.72	87.13	16.98	-0.02	0.9774	0.9488, 1.0070
peak	492	433.45	5.22	0.73	0.12	0.73	0.12	0.73	42.68	38.98	62.03	0.00	0.9952	0.9681, 1.0231
$TAUC_{16}$	484	396.48	17.28	0.03	2.55	0.11	2.61	0.01	79.34	88.19	26.09	0.02	1.0209	0.9956, 1.0469
$TAUC_{40}$	485	400.29	6.94	0.54	0.07	0.79	0.07	0.79	85.36	99.76	0.00	0.00	1.0049	0.9691, 1.0421

HIV RNA Level (ritonavir subjects, no baseline variables)

	n	-2LL	HL χ^2	sig	Model χ^2	sig	Wald	sig	%correct	spec	sens	β	OR	95% CI
Baseline	87	76.03	12.72	0.12	3.96	0.05	3.18	0.07	55.17	48.61	86.67	1.33	3.7795	0.8771, 16.2856
week 2	75	72.14	8.90	0.26	0.06	0.80	0.06	0.80	18.67	0.00	100.00	-0.08	0.9215	0.4830, 1.7581
week 4	73	68.28	8.30	0.41	0.12	0.74	0.11	0.74	28.77	18.33	76.92	0.10	1.1039	0.6206, 1.9636
week 8	75	70.03	7.89	0.34	2.17	0.14	1.81	0.18	42.67	32.79	85.71	0.43	1.5410	0.8210, 2.8927
week 12	69	70.66	4.54	0.81	1.60	0.21	1.35	0.25	36.23	20.37	93.33	0.45	1.5689	0.7338, 3.3543
week 16	72	71.50	10.08	0.26	2.19	0.14	1.70	0.19	34.72	19.30	93.33	0.59	1.8016	0.7431, 4.3679
peak	79	76.59	19.06	0.01	0.21	0.65	0.20	0.65	24.05	7.81	93.33	0.13	1.1333	0.6583, 1.9511
$TAUC_{16}$	74	62.21	7.28	0.51	0.59	0.44	0.65	0.42	70.27	80.95	9.09	-0.18	0.8318	0.5317, 1.3013
$TAUC_{40}$	74	60.38	10.68	0.22	1.83	0.18	1.36	0.24	52.70	52.38	54.55	0.79	2.2115	0.5839, 8.3752

Appendix 6

Adjusted Univariate Logistic Regression: Ritonavir Subjects

CD4 (ritonavir subjects, with adjustment for baseline variables)

	n	-2LL	HL:χ^2	sig	Modelχ^2	sig	Wald	sig	%correct	spec	sens	β	OR	95% CI
Baseline	494	427.22	11.26	0.19	13.61	0.00	4.99	0.03	50.20	46.00	71.60	-0.01	0.9866	0.9749, 0.9983
week 2	427	371.71	12.82	0.12	6.02	0.11	0.62	0.43	44.26	39.66	68.12	0.00	0.9975	0.9913, 1.0038
week 4	428	364.55	4.74	0.79	13.53	0.00	5.18	0.02	53.27	50.42	68.12	-0.01	0.9938	0.9884, 0.9991
week 8	422	346.61	9.45	0.31	19.39	0.00	9.44	0.00	58.06	55.90	69.70	-0.01	0.9902	0.9840, 0.9964
week 12	390	321.06	23.16	0.00	17.20	0.00	9.15	0.00	56.15	55.02	62.30	-0.01	0.9902	0.9838, 0.9965
week 16	378	287.71	7.45	0.49	18.73	0.00	6.79	0.01	62.17	61.23	67.92	-0.01	0.9906	0.9837, 0.9977
peak	479	409.12	5.29	0.73	23.05	0.00	10.60	0.00	55.32	51.88	72.50	-0.01	0.9923	0.9877, 0.9969
$TAUC_{16}$	466	390.83	7.46	0.49	20.49	0.00	9.35	0.00	58.80	56.68	71.01	-0.01	0.9872	0.9790, 0.9954
$TAUC_{40}$	467	364.11	5.77	0.67	27.04	0.00	13.21	0.00	60.17	58.54	69.57	-0.02	0.9828	0.9736, 0.9920

CD4% (ritonavir subjects, with adjustment for baseline variables)

	n	-2LL	HLχ^2	sig	Modelχ^2	sig	Wald	sig	%correct	spec	sens	β	OR	95% CI
Baseline	475	412.19	7.57	0.48	12.07	0.01	4.40	0.04	51.16	47.86	67.95	-0.10	0.9058	0.8258, 0.9935
week 2	412	360.71	2.00	0.98	5.14	0.16	0.05	0.82	40.29	32.75	79.10	0.01	1.0076	0.9441, 1.0753
week 4	412	352.01	6.02	0.65	10.55	0.01	3.92	0.05	52.43	49.71	66.67	-0.09	0.9140	0.8361, 0.9991
week 8	409	347.63	2.47	0.96	10.56	0.01	4.65	0.03	42.32	50.00	64.62	-0.11	0.8940	0.8075, 0.9899
week 12	382	320.66	10.46	0.23	11.51	0.01	5.89	0.02	56.81	54.97	66.67	-0.12	0.8848	0.8015, 0.9768
week 16	368	283.85	3.55	0.90	15.94	0.00	6.34	0.01	63.04	63.61	59.62	-0.14	0.8687	0.7786, 0.9693
peak	461	406.14	18.79	0.02	9.81	0.02	2.35	0.13	47.29	42.97	68.83	-0.05	0.9543	0.8989, 1.0131
$TAUC_{16}$	447	370.72	8.77	0.37	13.58	0.00	6.88	0.01	66.00	69.63	44.62	-0.09	0.9170	0.8652, 0.9522
$TAUC_{40}$	449	362.95	11.71	0.16	15.43	0.00	7.58	0.01	57.68	57.07	61.19	-0.18	0.8351	0.7346, 0.9494

CD8 (ritonavir subjects, with adjustment for baseline variables)

	n	-2LL	HLχ^2	sig	Modelχ^2	sig	Wald	sig	%correct	spec	sens	β	OR	95% CI
Baseline	481	418.84	10.90	0.21	7.56	0.06	0.63	0.43	42.20	35.98	74.36	0.00	0.9997	0.9988, 1.0005
week 2	411	357.69	6.31	0.61	4.51	0.21	0.23	0.63	41.85	36.23	71.21	0.00	0.9997	0.9983, 1.0010
week 4	411	355.79	6.82	0.56	6.41	0.09	0.49	0.49	44.77	39.42	72.73	0.00	0.9998	0.9992, 1.0004
week 8	407	339.46	6.18	0.63	14.70	0.00	7.80	0.01	57.00	45.39	65.63	0.00	0.9990	0.9983, 0.9997
week 12	380	321.27	8.98	0.34	10.21	0.02	4.73	0.03	53.16	50.31	68.33	0.00	0.9992	0.9984, 0.9999
week 16	366	284.44	4.96	0.76	11.12	0.01	3.09	0.08	61.75	61.90	60.78	0.00	0.9992	0.9983, 1.0001
peak	463	403.44	8.50	0.39	13.24	0.00	5.18	0.02	52.48	48.70	71.43	0.00	0.9993	0.9988, 0.9999
$TAUC_{16}$	454	376.46	13.92	0.08	6.55	0.09	0.41	0.52	58.81	62.11	39.39	0.00	1.0002	0.9996, 1.0008
$TAUC_{40}$	454	359.81	4.82	0.78	16.65	0.00	8.84	0.00	60.13	59.28	65.15	0.00	0.5137	0.2630, 1.0031

CD8% (ritonavir subjects, with adjustment for baseline variables)

	n	-2LL	HL χ^2	sig	Model χ^2	sig	Wald	sig	%correct	spec	sens	β	OR	95% CI
Baseline	475	417.06	3.52	0.90	7.20	0.07	0.15	0.70	39.79	31.49	82.05	0.00	0.9970	0.9817, 1.0125
week 2	412	359.91	9.56	0.30	5.94	0.11	0.84	0.36	46.84	42.32	70.15	0.02	1.0190	0.9788, 1.0609
week 4	412	356.63	8.02	0.43	5.92	0.12	0.96	0.81	40.53	32.95	80.30	0.00	1.0038	0.9736, 1.0349
week 8	409	352.81	5.09	0.75	5.38	0.15	0.00	0.99	40.83	33.43	80.00	0.00	0.9998	0.9743, 1.0260
week 12	382	327.01	3.20	0.92	5.16	0.16	0.49	0.48	44.50	38.82	75.00	-0 01	0.9900	0.9626, 1.0182
week 16	368	288.27	9.52	0.30	11.52	0.01	3.02	0.08	62.23	64.24	50.00	-0 03	0.9727	0.9428, 1.0035
peak	461	408.58	7.30	0.51	7.38	0.06	0.15	0.70	40.35	32.03	81.82	-0 01	0.9944	0.9666, 1.0229
$TAUC_{16}$	453	379.66	10.36	0.24	9.26	0.03	2.92	0.09	54.75	54.66	55.22	0.02	1.0225	0.9968, 1.0490
$TAUC_{40}$	454	376.77	11.33	0.18	6.71	0.08	0.00	0.96	39.65	32.12	82.35	0.00	1.0009	0.9640, 1.0392

HIV RNA Level (ritonavir subjects, with adjustment for baseline variables)

	n	-2LL	HL χ^2	sig	Model χ^2	sig	Wald	sig	%correct	spec	sens	β	OR	95% CI
Baseline	82	69.53	11.29	0.19	8.50	0.04	4.26	0.04	62.20	58.21	80.00	1.65	5.2056	1.0862, 24.9463
week 2	71	67.12	7.65	0.47	3.38	0.34	0.07	0.80	59.15	61.40	50.00	-0.09	0.9155	0.4655, 1.8006
week 4	68	64.39	8.82	0.36	1 97	0.58	0.13	0.72	48.53	45.45	61.54	0.11	1.1132	0.6175, 2.0067
week 8	70	65.40	10.89	0.21	4.66	0.20	2.46	0.12	54.29	46.43	85.71	0.51	1.6619	0.8806, 3.1364
week 12	65	66.32	6.57	0.48	3.91	0.27	0.86	0.35	41.54	26.00	93.33	0.36	1.4396	0.6673, 3.1058
week 16	67	67.32	3.88	0.87	3.94	0.27	1.14	0.29	35.82	23.08	80.00	0.49	1.6306	0.6635, 4.0078
peak	74	71.47	6.08	0.64	3.14	0.37	0.21	0.64	45.95	40.68	66.67	0.13	1.1422	0.6503, 2.0063
$TAUC_{16}$	70	60.89	3.70	0.88	1.89	0.60	0.34	0.56	71.43	77.97	36.36	-0.13	0.8713	0.5473, 1.3870
$TAUC_{40}$	70	57.49	10.66	0.22	3.40	0.33	1.39	0.24	61 43	61.02	63.64	0.84	2.3205	0.5723, 9.4092

Appendix 7

Adjusted Univariate Logistic Regression: Placebo Subjects

CD4 (placebo subjects, adjusted for baseline variables)

	n	-2LL	HLχ^2	sig	Modelχ^2	sig	Wald	sig	%correct	spec	sens	β	OR	95% CI
Baseline	472	588.65	12.68	0.12	12.55	0.01	9.22	0.00	51.27	43.34	68.46	-0.01	0.9874	0.9793, 0.9955
peak	471	587.89	9.39	0.31	3.51	0.32	1.29	0.26	51.80	46.89	62.42	0.00	1.0042	0.9970, 1.0114
$TAUC_{16}$	457	559.85	7.85	0.45	4.82	0.19	1.85	0.17	56.02	56.43	55.07	0.01	1.0112	0.9951, 1.0276
$TAUC_{40}$	458	560.56	8.27	0.41	3.69	0.30	0.69	0.41	54.15	52.50	57.97	0.01	1.0057	0.9923, 1.0192

CD4% (placebo subjects, adjusted for baseline variables)

	n	-2LL	HLχ^2	sig	Modelχ^2	sig	Wald	sig	%correct	spec	sens	β	OR	95% CI
Baseline	454	564.14	4.45	0.81	5.41	0.14	1.46	0.23	53.08	47.12	66.20	-0.04	0.9617	0.9025, 1.0247
peak	453	563.39	8.47	0.39	5.07	0.17	1.07	0.30	47.02	36.33	70.42	0.04	1.0428	0.9631, 1.1291
$TAUC_{16}$	440	534.13	12.04	0.15	4.91	0.18	0.10	0.76	52.50	49.68	59.23	0.02	1.0162	0.9175, 1.1255
$TAUC_{40}$	438	529.23	7.55	0.48	5.63	0.13	0.35	0.56	53.20	50.32	60.16	0.05	1.0473	0.8981, 1.2213

Appendix 8

Comparison of Specificities of Multiple Logistic Regressions

Entry 1 (Covariates entered: CD4, CD4%, CD8, CD8%, Viral Load)

All Subjects

	Model	n	Specificity
Baseline	---		
Week 2	CD8	129	33.33
Week 4	viral load	129	32.26
Week 8	CD8, CD8%, viral load	125	40.00
Week 12	---		
Week 16	---		
Peak	CD8	137	33.67
TAUC16	CD4	128	47.92
TAUC40	CD4	127	48.96

Ritonavir Subjects

	Model	n	Specificity
Baseline	viral load	82	58.21
Week 2	---		
Week 4	---		
Week 8	CD4	70	42.86
Week 12	---		
Week 16	CD8%	63	60.78
Peak	CD8	73	46.55
TAUC16	---		
TAUC40	CD8	68	58.82

(model = covariates included in final model)

(n = number of subjects included in analysis)

(specificity = specificity of final model)

Comparison of Specificities of Multiple Logistic Regressions

Entry 2 (Covariates entered: CD4, CD8, Viral Load)

All Subjects

	Model	n	Specificity
Baseline	---		
Week 2	CD8	129	33.33
Week 4	viral load	129	32.26
Week 8	CD8, viral load	125	37.78
Week 12	---		
Week 16	CD8	110	41.77
Peak	CD8	137	33.67
TAUC16	CD4	128	47.92
TAUC40	CD4	128	50.00

Ritonavir Subjects

	Model	n	Specificity
Baseline	viral load	82	58.21
Week 2	---		
Week 4	---		
Week 8	CD4	70	42.86
Week 12	---		
Week 16	---		
Peak	CD8	73	46.55
TAUC16	---		
TAUC40	CD8	68	58.82

Comparison of Specificities of Multiple Logistic Regressions
Entry 3 (Covariates: CD4, CD4%, CD8, CD8%)

All Subjects

	Model	n	Specificity
Baseline	CD4	928	49.22
Week 2	CD4	836	44.58
Week 4	CD4	832	49.37
Week 8	CD4%	803	54.60
Week 12	CD4	756	50.17
Week 16	CD4	714	55.40
Peak	CD4	913	50.94
TAUC16	CD4, CD4%, CD8	881	56.12
TAUC40	CD4	881	52.47

Ritonavir Subjects

	Model	n	Specificity
Baseline	CD4	475	49.89
Week 2	---		
Week 4	CD4	411	50.43
Week 8	CD4	407	55.69
Week 12	CD4	380	55.63
Week 16	CD4%, CD8%	366	54.64
Peak	CD4	461	52.34
TAUC16	CD4, CD4%, CD8	444	58.01
TAUC40	CD4	446	60.00

Comparison of Specificities of Multiple Logistic Regressions

Entry 4 (Covariates: CD4, CD4%, CD8, CD8%)

All Subjects

	Model	n	Specificity
Baseline	--		
Week 2	CD8	132	33.68
Week 4	CD8	132	25.53
Week 8	CD8, CD8%	128	38.71
Week 12	CD4	117	24.39
Week 16	CD8	110	41.77
Peak	CD8	138	33.33
TAUC16	CD4	141	50.93
TAUC40	CD4	140	50.93

Ritonavir Subjects

	Model	n	Specificity
Baseline	--		
Week 2	CD4%, CD8	70	52.73
Week 4	CD8	71	39.29
Week 8	CD4	71	43.86
Week 12	--		
Week 16	CD8%	63	60.78
Peak	CD8	73	46.55
TAUC16	CD4, CD4%	75	60.00
TAUC40	CD8	75	60.00