EFFECTIVENESS OF HIGHLY ACTIVE ANTIRETROVIRAL THERAPY IN HIV PATIENTS IN RESOURCE-LIMITED SETTINGS

by

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The HIV pandemic has posed an unprecedented challenge to the global health, devastating communities and reinforcing the historical problems that link ill-health with poverty. Sound evidence for public health decision-making is needed as antiretroviral programmes are being rolled out in developing countries. This thesis discusses the effectiveness of Highly Active Antiretroviral Therapy (HAART) in resource-limited countries from the Antiretroviral Therapy in Lower Income Countries (ART-LINC), a network of HIV/AIDS treatment programmes and cohorts in Africa, South America, and Asia. The objectives of this project were to document the effectiveness of HAART in these settings, defined by changes in immunologic and virologic markers within 6 months of treatment; assess factors associated with 6-month response to therapy; and to assess the association of 6 month response with long-term outcomes.

In the first article, the evidence supporting effectiveness of HAART is reviewed, focusing on aspects of immunologic and virologic responses to therapy. Despite the lack of a standardized definition of immunologic and virologic response, we conclude that around one third of the patients who start HAART show a response pattern where either immunologic or virologic response is not achieved, a condition referred to as discordant response.

The second and third articles provide a picture of the association between HAART and 6-month response in resource-limited countries. Overall, the effectiveness of HAART in these settings is similar to that reported in resource-rich countries. Finally, we assessed the association

between immunologic and virologic discordant responses at 6 months and mortality in ART-LINC. We found that the hazard of death for those showing discordance at 6 months was similar to that reported in resource-rich countries. However, we found that early mortality was high in Africa and Asia, and a significant proportion of patients that did not have access to laboratory measurements were also at greater risk of death. This is the first report on the association of discordant responses and mortality in lower income countries, which provides important evidence for public health decision making in the context of antiretroviral rollout.

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1.0 BACKGROUND AND LITERATURE REVIEW

1.1 EFFICACY AND EFFECTIVENESS OF HAART IN DEVELOPED COUNTRIES

In industrialized countries, the widespread use of highly active antiretroviral therapy (HAART) has led to major reductions in morbidity and mortality associated with HIV infection [1-6]. The initiation of HAART rapidly and significantly reduces the levels of virus replication in the peripheral blood, and increases levels of CD4 count [7, 8], two important predictors of disease progression.

In randomized controlled trials, HAART was shown to reduce plasma viral load (PVL) to less than 500 copies/Ml in 60 to 90% of the patients [5-8]. A meta-analysis of 23 clinical trials involving 31 independent treatment groups, 19 unique antiretroviral regimens, and 3,257 enrolled treatment naïve patients, showed that 64% (95% Confidence Interval [CI] 60 – 67) of patients taking HAART showed viral suppression (PVL<400 copies/Ml) at 24 weeks [9].

Observational studies, which better reflect the clinical practice, have shown a variety of results, using different thresholds and timeframes to define virologic response. Table 1 shows virologic response in several observational studies reported in the literature.

Table 1. Virologic response in various observational studies from developed countries

Author	N	Study Population	Definition of virologic success (log_{10} or copies/mL)	Virologic success rate (%)
Mocroft et al.[10]	243	PI naïve, including pre-treated patients	PVL<400 copies at 6 months	52.8
Lucas et al. [11]	273	PI naive, including pre-treated patients	PVL<500 copies at 1–90 days, and 3–7 and 7–14 months	42.0 at 1–90 days; 44.0 at 3–7 months; and 37.0 at 7–14 months

Table 1 continued:

Author	N	Study Population	Definition of virologic success (log_{10} or copies/mL)	Virologic success rate (%)
Ledergerber et al. [12]	2674	Pre-treated and naive patients	PVL<400 copies by 12 months	90.7 of treatment naive and 70.3–78.7 of pre-treated
Staszewski et al. [13]	901	PI naive, including pre-treated patients	PVL<500 copies at 6 months	79.0
Paredes et al. [14]	1469	HAART naïve, including pre-treated patients	PVL<500 copies at 6 months	60.4
Grabar et al. [15]	1402	PI naive, mostly (>80%) pre-treated patients	PVL<1000 copies at 12 months	50.0
Le Moing et al. [16]	1129	PI naive, including pre-treated patients	PVL<500 copies at 4 months	73.5
Pulido et al. [17]	310	HAART naïve, severely immunodepressed patients (CD4 cell count<100)	PVL<400 copies at 6 months	Efavirenz recipients: 74.4; PIs recipients: 57.8

PI: Protease Inhibitor

PVL: Plasma Viral Load

More important, studies have shown that early patterns of change in both virologic and immunologic responses to HAART predict progression to AIDS or death, independent of pre-treatment clinical or laboratory parameters. For example, among 1,132 HIV-infected women from the Women's Interagency HIV Study (WIHS), Anastos et al. found that CD4+ cell count and PVL attained after treatment initiation were stronger predictors of clinical progression and death than pre-treatment values [18]. Another study of 2,236 HIV patients who started on HAART with 1 PI in France found that patients showing a virologic only response at 6 months were at higher risk of clinical progression or death than those showing a complete response [15]. Several studies, using different definitions, have documented the frequency, risk factors and long-term outcomes associated with discordant responses in developed countries.

1.2 ARTICLE 1 – DISCORDANT IMMUNOLOGIC AND VIROLOGIC RESPONSES TO ANTIRETROVIRAL THERAPY

(From manuscript originally published in the Journal of Antimicrobial Chemotherapy 58: 506 -10, 2006)

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Key words: CD4 lymphocyte count; viral load; treatment outcome

1.2.1 ABSTRACT

In response to antiretroviral therapy, some patients experience what has been termed a discordant response, characterized either by a sustained CD4+ cell count rise despite persistent viraemia or by HIV-1 RNA plasma levels below the limit of detection accompanied by a blunted CD4+ cell count response. In part because of a lack of universally accepted definitions, published estimates of the frequency of discordant responses vary considerably. Little is known about the pathogenesis of discordant responses, which seems to depend on the interaction of a multitude of viral, host, and treatment-related factors. Available evidence indicates that discordant responses are associated with an intermediate risk of death or clinical progression. At present, recommendations for the clinical management of patients with discordant responses to antiretroviral therapy are largely based on observational, uncontrolled data. The development of standardized and universally accepted definitions of discordant responses is necessary to allow meaningful comparisons between studies to be made, as well as to help in the design trials of possible therapeutic interventions.

1.2.2 INTRODUCTION

The introduction of highly active antiretroviral therapy (HAART) into clinical practice has led to dramatic reductions in morbidity and in mortality associated with infection with the human immunodeficiency virus (HIV). The initiation of HAART generally leads to a rapid reduction in HIV-1 RNA plasma levels and to an increase in peripheral CD4+ cell counts[5, 7, 8]. However, some patients experience a "discordant response", whereby the HIV-1 RNA plasma level is below the limit of detection but the CD4+ cell count response is blunted. Other patients exhibit a different pattern of discordant response, characterized by a sustained CD4+ cell count response, despite persistent viraemia. In this article, we discuss the epidemiology, risk factors, prognosis, and recommendations for the clinical management of patients with discordant responses.

1.2.3 EPIDEMIOLOGY

It is not surprising that, given the lack of universally accepted definitions of virologic and immunologic success, published estimates of the frequency of discordant responses vary considerably (Table 2). Definitions of virologic success that have been used include HIV-1 RNA plasma levels below 50, 400 or 1,000 copies/mL, whereas definitions of immunologic response include increases in CD4+ cells counts above a certain value (usually 50 cells at the end of the first year of therapy) or maintenance of CD4+ cell counts above a certain threshold (generally 200 cells/mm^3).

Table 2. Discordant responses in various observational studies

Author	N	Study Population	Definition of immunologic (cells/mm^3) and of virologic responses (log_{10} or copies/mL)	Proportion of discordant responses (%)	
				Virologic only	Immunologic only
Piketty *et al.*	162	PI naïve, previously NRTI experienced	↑ CD4+>50 and ↓ PVL>1 log_{10} over mean follow-up of 12.1 months	10.5	10.5
Grabar *et al.*	2,236	PI naïve, including pre-treated patients	↑ CD4+>50 and ↓ PVL>1 log_{10} at 6 months	17.3	19.0
Nicastri *et al*.	2,143	PI naïve, including pre-treated patients	↑ CD4+>100 and PVL<500 at 12 months	15.7	20.6
Mehta *et al.*	265	Injecting drug users initiating HAART, including pre-treated patients	↑ CD4+>50 or CD4+>500 and PVL<1,000 at 12 months	16.0	21.0
Moore *et al.*	2,217	Previously treatment naïve patients initiating HAART	↑ CD4+>50 and PVL<500 at 6 months	15.4	11.7
Marimoutou *et al.*	478	Patients initiating HAART with PI, including pre-treated patients	↑ CD4+>50 and PVL<500 at 6 months	15.7	23.8
Schechter *et al.*	1,916	Previously treatment naïve patients initiating HAART	↑ CD4+>50 and PVL<500 at 6 months	14.0	19.0

In industrialized countries, discordant responses have been reported to occur in 20 to 30% of patients 6 months to 2 years after starting therapy. For example, in a cohort of 162 HIV patients receiving indinavir and followed for a median of 10.5 months, Piketty *et al.* found equal rates (10.5%) of immunologic only, and virologic only responses. The authors defined immunologic success as an increase of 50 CD4+ cells/mm^3 and virologic success as either a decrease in HIV RNA plasma viral load by 1 log_{10} or achieving a plasma viral load < 400 copies/mL at any point during the follow-up period (median 12 months, range 2 to 15 months) [19]. In another study, conducted in France, immunologic success was defined as an increase of CD4+ > of more than 50 cells/mm^3 and virologic success as a decrease in HIV RNA viral load of more than 1 log_{10} copies/mL, after 6 months on treatment. In 2,236 protease inhibitor (PI) naïve patients studied

19% exhibited immunologic only response, whereas 17.3% had virologic only response[15]. In a study conducted in Italy, which included 3,094 anti-retroviral naïve and experienced patients, 20.6% were reported to have immunologic only responses and 15.7% to have virology only response. The definitions used in this study were an increase of at least 100 CD4+ cells/mm^3 and plasma viral load levels below 500 copies/mL at 12 months[20]. In a cohort of 265 injecting drug users (IDU) in Baltimore, Maryland, immunologic and virologic only responses were observed in 21 and 16%, respectively. Virologic response was defined as a viral load < 1,000 copies/mL and immunologic response as either an increase of 50 CD4+ cells or achieving CD4+ >500 cells/mm^3 at the end of the first year on HAART[21]. In a study conducted in British Columbia, Canada, an increase in 50 CD4+ cells/μL and achieving viral load<500 copies/mL at 6 months on therapy were used to define successful immunologic and virologic responses, respectively. The study involved 1,527 treatment-naive patients and found that 11.7% and 15.4% of the subjects showed immunologic and virologic only responses, respectively[22].

There are limited data on discordant responses in patients being treated in developing countries. The Antiretroviral Therapy in Lower Income Countries Collaboration (ART-LINC), an epidemiologic network of HIV/AIDS treatment programs in Africa, Asia, and South America, has reported on the frequency of discordant responses in 1,916 previously treatment-naïve patients seen in 15 developing countries who initiated HAART between March 1996 and April 2004. 269 (14%) were virologic only and 365 (19%) were immunologic only responders [23] with virologic response defined as achieving a plasma HIV RNA viral load < 500 copies/mL, and immunologic response defined as an increase of at least 50 CD4 cells/mm^3 at 6 months.

1.2.4 RISK FACTORS

Little is known about the pathogenesis of discordant responses, which seems to depend on the interaction of a multitude of viral, host, and treatment-related factors.

A blunted CD4 response despite suppression of viral replication has often been attributed to host characteristics, particularly older age[15, 22, 24-26]. It has been hypothesized that the magnitude of immune restoration is dependent on thymus activity, which decreases with age[27]. Poor CD4 cell reconstitution despite virologic response has also been correlated with a lower nadir pre-treatment CD4+ cell count, suggestive of more extensive depletion of CD4+ cells in the gut-associated lymphoid tissue during primary (acute) HIV infection, which may be slow or refractory to reconstitution with antiretroviral therapy[28, 29]. Moreover, it has been suggested that the rate of CD4+ cell depletion after HIV infection is established is also important: slower rates of decrease in CD4+ cell counts before therapy initiation are associated with a slower early recovery, whereas sharper slopes of CD4+ cell decrease with maximal subsequent cell distribution once therapy is instituted[30].

Genetic variability has also been investigated as a possible modulator of immunologic recovery. An example is the multidrug-resistance transporter gene MDR1, which codes for P-glycoprotein, an essential protein involved in transportation of many different substrates, including antiretroviral drugs. Overexpression of P-glycoprotein lowers intracellular concentration of protease inhibitors[31] and could therefore affect reconstitution of CD4+ cell pool. Association of MDR1 polymorphism (3435 TT genotype) with improved CD4 recovery was found in two studies,[32, 33] but not in another[34]. Polymorphisms within the CCR5 gene, which codes for a key cell surface co-receptor for macrophage-tropic strains of HIV-1, have long been associated with different patterns of susceptibility to infection [35] and disease progression

[36]. However, it appears that such polymorphisms do not have a significant impact on initial virological and immunological response to antiretroviral therapy [37, 38]. Finally, a few studies have looked at genes that are associated with T lymphocyte apoptosis. Combination of some polymorphisms of Fas and Fas ligand (FasL), two important genes that control T lymphocyte homeostasis [39], and carriage of a specific allele that encodes IL-6 production [38] have been implicated on altered CD4+ recovery.

Other factors that have been reported associated with blunted CD4+ response are infection by HIV-2[40], co-infection by HTLV-1 (human T-lymphotropic virus type I), or HTLV-2, and due to drug toxicity. The use of the combination tenofovir + didanosine [41, 42] has been implicated in failure to increase CD4+ cell count despite of viral suppression, and this effect is apparently dependent on doses of didanosine. The underlying mechanism is thought to be a synergistic effect of both tenofovir and didanosine metabolites in producing an imbalance in the purine pool within CD4+ T lymphocytes, which, in turn, has a cytostatic effect on these cells [43].

The use of zidovudine or didanosine, as part of the antiretroviral regimen, both which can cause lymphopenia, the concurrent use of other myelotoxic drugs such as co-trimoxazole, or the presence of certain co-infections, such as HTLV-1, have all been associated with sub-optimal CD4+ cell responses despite suppression of viral replication.

The physiology of a good immunologic response in the presence of virologic failure is also not well understood and possibly involves various aspects of virus-drug-host dynamics. In one closely followed cohort of 402 patients who had laboratory evaluations repeatedly in the first year after initiation of HAART, all but 2 of the 25 immunologic only responders had either a prior transient period of undetectable plasma viraemia or, at least, HIV-1 RNA plasma levels

below <1000 copies/mL suggesting that temporary or partial viral suppression was the primary mechanism involved in the observed increases in CD4+ cell counts [44]. One possible explanation is that HAART selects for viral strains with lower replicative capacity and reduced pathogenicity. The observation that virus isolated from recipients of protease inhibitor (PI)-based regimens with stable CD4+ cell counts despite virologic failures have decreased viral replication capacity seems to corroborate this hypothesis[45, 46]. In one study, lower replication capacity, or reduced fitness, was associated with the presence of non-syncytium-inducing viral phenotype, decreased cellular activation, and enhanced interferon-γ production in response to *gag* and *tat* antigens in the setting of extensive genotypic and phenotypic resistance to PIs [46]. Patients in this study exhibited sustained or even increased CD4+ counts for periods exceeding 5 years without disease progression, despite extensive and stable antiretroviral resistance profile[47]. The sustained increase in CD4+ cell count in the context of prolonged virologic failure may be explained by a state of relative immunologic quiescence, in which T cell dynamics are similar but not identical to what occurs in complete responders, in which the turnover of CD4+ cells has been shown to be slower, denoted by their reduced expression of markers of activation ($CD38^+$, $HLA\text{-}DR^+$) and active cycling ($Ki67^+$), compared to untreated patients [48].

Antiretroviral resistance mutations that are associated with reduced replication capacity have been frequently reported in patients with immunologic response despite virologic failure. Although both immunologic only responders and non-responders seem to share a similar resistance pattern, a higher percentage of viral strains carrying the M184V mutation, associated with lamivudine resistance, has been reported in immunologic only responders than in non-responders [49-51]. In one study of patients who remained on a stable PI-based regimen despite detectable plasma viraemia, the emergence of primary mutations V82A, I84V and/or L90M were

associated with a decreased replicative capacity [52]. In another study in patients who had failed HAART, the M36I mutation was only found in immunologic only responders [51].

There are no published controlled studies evaluating the impact of different antiretroviral regimens on the incidence of discordant responses. On the other hand, published observational studies mostly include patients receiving PI-based regimens. In the observational study of Moore et al, no association was found between type of regimen and presence of discordant responses 3 to 9 months after HAART initiation [22]. Nonetheless, there are data to indicate that the degree of impairment in viral replicative capacity in virus isolated from patients with discordant responses may depend on the type of antiretroviral regimen being used, replicative capacity being higher in patients on non-nucleoside reverse transcriptase inhibitor-based regimens than in patients receiving PI- based regimens, perhaps reflecting different barriers to selection of resistant virus [53]. There are also data to indicate that in patients with sustained CD4+ cell counts despite prolonged virologic failure, interruption of the PI while maintaining the reverse transcriptase inhibitor backbone had little impact on CD+ cell counts or plasma viraemia, whereas the reverse led to significant increases in HIV-1 RNA plasma levels and decreases in CD4+ cell counts [48].

Adherence to therapy may also influence the occurrence of discordant responses. In the study of Moore et al, sub-optimal adherence was found to be associated both with virologic and immunologic only responses [22].

1.2.5 PROGNOSIS

The use of different definitions of virologic and immunologic responses, as well as different follow-up periods, do not allow for direct comparisons between the various studies that

attempted to determine the prognostic impact of discordant responses. Nonetheless, the majority of published studies have indicated that, in comparison to complete response, discordant responses are associated with an intermediate risk of death or clinical progression.

In the previously cited study of Grabar *et al.*, virologic only responders and non-responders had a higher probability of clinical progression, whereas immunologic only and complete responders had similar risks [15]. In contrast, in other studies immunologic only response was also associated with a higher risk of clinical progression. In one cohort of antiretroviral experienced patients with advanced HIV disease starting PI based HAART, who were followed for over 30 months, discordant responders at 12 months experienced significantly more AIDS-defining events than complete responders, with immunologic only responders presenting a slightly higher probability of being event-free compared to virologic only responders [25]. In another study involving over 2,100 antiretroviral experienced and naïve HIV patients followed for a median of 44 months, immunologic only and virologic only responders had a significantly lower risk of clinical progression than non-responders, but a 2.3 and 1.9-fold greater risk of death or of experiencing a new AIDS-defining event than complete responders, respectively [15].

Very few studies have assessed the prognostic impact of discordant responses in naïve patients and in recipients of NNRTI-base regimens. In the study of Moore *et al.*, mortality was increased in patients showing an early discordant response, but no statistically significant difference was found between immunologic and virologic only responders [22]. Likewise, in a study involving HAART naïve IDU patients, discordant responders had an increased mortality compared to complete responders, but progression rates did not differ by whether early response was immunologic or virologic only [21]. We are not aware of any published study assessing the prognostic impact of discordant responses in low-income settings.

1.2.6 CLINICAL MANAGEMENT

At present, recommendations for the clinical management of patients with discordant responses to antiretroviral therapy are largely based on observational, uncontrolled data. For patients on a new regimen, who after several months of treatment the HIV-1 RNA level is below the limit of detection but the CD4+ cell count response is blunted, it is recommended to maintain the current regimen. Changing or intensifying the regimen has not been shown to have an effect on the CD4+ cell count response, except in the case of patients whose regimen contains antiretroviral drugs that are associated with lymphopenia, such as zidovudine or didanosine. One controlled study showed that concomitant administration of human recombinant growth hormone may be beneficial in enhancing immunologic response chronically infected patients [54]. If the patient is using concomitant medications associated with bone marrow toxicity, their interruption or substitution should be judiciously considered.

Given that no benefit has so far been demonstrated and the potential for significant toxicity, the use of immune modulators, such as interleukin-2, is not recommended, except in the setting of clinical trials. For patients with a sustained CD4+ cell count response, despite persistent viraemia, the goal of therapy should remain the suppression of HIV-1 RNA to levels below 50 copies/mL. A detailed assessment of adherence, drug intolerance and pharmacokinetic issues should be done to rule out modifiable causes. If none is encountered, then modifications in the antiretroviral regimen should be considered, the choice of drugs being dependent upon patient history and resistance testing. For patients who have previously failed multiple regimens, it may not be feasible to achieve plasma HIV-1 RNA levels of less than 50 copies/mL. For these patients, stability of CD4+ cell counts becomes the objective of treatment.

1.2.7 CONCLUSIONS

There are limited information on the pathogenesis, risk factors, prognosis and clinical management of discordant immunologic and virologic responses to antiretroviral therapy, despite its relative frequency. The development of standardized and universally accepted definitions of discordant responses is necessary to allow meaningful comparisons between studies to be made, as well as to help in the design trials of possible therapeutic interventions.

1.3 EFFECTIVENESS OF HAART IN RESOURCE-LIMITED SETTINGS

Although well documented in high income countries, data on response to therapy in resource-limited countries are limited. According to the 2007 report on the scaling up antiretroviral therapy strategy by the World Health Organization (WHO), in 2006, almost 700,000 people received treatment for the first time, and by December 2006 it was estimated that 2,015,000 (1.8−2.2 million) people living with HIV/AIDS were receiving treatment in low- and middle-income countries, representing 28% (24%−34%) of the estimated 7.1 million (6.0−8.4 million) [55]. The urgent need for quickly scaling up HAART has posed a complex challenge to the design and assessment of public health interventions as controlled trials cannot be used in such circumstances for ethical reasons. Evidence base, however, is necessary to demonstrate effect, assure a rational utilization of, and secure future funding of such interventions.

Data from observational studies suggest that virological and immunological efficacy of HAART appears to be as good as that observed in developed countries [56-58]. In a meta-analysis of 10 observational studies, HIV RNA viral load suppression was found in approximately 60-70% of individuals at time points up to month 18 [57].

With the rollout of HAART in developing countries, a high priority has been put into conduct research on its operational aspects, in particular to identify appropriate models of care and support to increase effective coverage in the long term [59-61]. It is also important that this research be timely and rooted in routine clinical management, and designed as multi-country studies that purposefully select countries with different relevant contextual factors, using hierarchical models to investigate the role of variables at each level [61].

1.3.1 The ART-LINC Collaboration

The Antiretroviral Therapy in Lower Income Countries (ART-LINC) Collaboration, a network of HIV/AIDS treatment programmes and cohorts in Africa, South America, and Asia was set up in 2003 to address these questions. The three primary objectives of ART-LINC are (i) to define the prognosis of HIV-1 infected patients treated with HAART in resource-limited settings; (ii) to compare the experience between different settings, delivery modes and types of monitoring; and (iii) to compare prognosis in resource-limited settings with that observed in industrialized nations. Importantly, both individual and programme level characteristics are of interest in this context. The current database includes patients from 15 countries from 3 continents: 3 countries North and Western Africa (Morocco, Côte d'Ivoire, and Senegal); 3 from Central and Eastern Africa (Kenya, Uganda, and Rwanda); 5 from Southern Africa (Botswana, Malawi, South Africa, Zambia, and Zimbabwe); 2 from South America (Argentina and Brazil), and 2 from Asia (India and Thailand) [62].

1.4 SPECIFIC AIMS

We here present 3 specific aims that address important aspects of HAART effectiveness in middle- and low-income countries, utilizing data from the ARTLINC collaboration.

Specific aim 1: to describe the virologic response in previously naïve patients starting HAART in a cohort of HIV infected patients in Porto Alegre, Brazil, and assesses risk factors for virologic failure at 6 months.

Specific aim 2: to assess the frequency of and risk factors for immunovirologic discordant responses in the ART-LINC collaboration.

Specific aim 3: to assess the long-term effect of early immunovirologic discordant responses on mortality in the ART-LINC collaboration.

2.0 ARTICLE 2 – VIROLOGIC RESPONSE IN PREVIOUSLY NAÏVE HIV PATIENTS INITIATING HAART IN PORTO ALEGRE, BRAZIL

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2.1 ABSTRACT

Objective: To assess predictors of virological response 6 months after initiation of highly active antiretroviral therapy (HAART) in a cohort of HIV infected patients in Brazil.

Methods: Treatment naïve patients who started HAART between 1996 and 2004 and had information on viral load at 3-9 months were included. Information was collected on demographic characteristics, antiretroviral regimen, adherence, AIDS diagnosis, baseline CD4 cell count, and viral load. Virological failure (VF) was defined as viral load $\geq$400 copies/mL at 6 months or death before completion of 6 months of therapy.

Results: Among 454 patients who met the inclusion criteria, VF occurred in 127 (28.0%). In univariate analysis, VF was associated with younger age [median 34 vs. 37, $p<0.01$], AIDS diagnosis (RR 1.18, $p<0.01$), higher baseline viral load [5.34 vs. 5.00, $p<0.01$], lower baseline CD4 cell count [86 vs. 182, $p<0.01$], non-adherence (RR 1.39, $p<0.01$), regimen containing 1 single protease inhibitor, as compared to ritonavir boosted regimens (OR 8.5, $p<0.01$), and year therapy initiated before 1999 ($p<0.01$). To minimize the systematic effect of therapy indication, we analyzed the subset of 158 patients with CD4 count $\leq$200 cells/uL who started therapy after 1999. After adjusting for age, education, adherence, regimen and baseline viral load, non-adherence (OR 8.78, $p=0.02$) and fewer years of education (OR 6.05, $p=0.05$) remained associated with VF.

Conclusions: We found a significant improvement in virological suppression over time, consistent with the introduction of non-nucleoside reverse transcriptase inhibitors and ritonavir boosted regimens into clinical practice. With currently available therapies, compliance and education were shown to be predictors of virological response, particularly in more immunocompromised patients.

2.2 INTRODUCTION

In developed countries, the widespread use of highly active antiretroviral therapy (HAART) has led to major reductions in morbidity and mortality associated with HIV infection [1, 2, 6, 7, 63]. In Brazil, since 1996 access to antiretroviral therapy has been universal and free of charge to all individuals who qualify for treatment according to national guidelines. Nonetheless, data on the response to HAART in Brazil are limited [64-68].

Studies conducted in developed countries have indicated that in therapy-naïve patients, virological response at 6 months of HAART is an important independent predictor of progression to AIDS or death, independent of pre-treatment clinical or laboratory parameters [15, 18, 69].

In the present study, we evaluated response to therapy in a cohort of treatment naïve HIV infected patients who started HAART between 1996 and 2004 in a public outpatient HIV clinic in Porto Alegre, Brazil.

2.3 METHODS

2.3.1 Study design and patients

This retrospective cohort study included all treatment-naïve, HIV-infected adults who attended the HIV/AIDS outpatient clinic at the Hospital de Clínicas de Porto Alegre, were prescribed HAART between January 1996 and January 2004, and for whom information was available on HIV PVL 3-9 months after treatment was initiated. This clinic is a government-owned facility where antiretroviral drugs and treatment are provided free-of-charge, and is a participating site in the ART-LINC collaboration.

To adjust for changes in clinical practices over time and for differences due to selection-by-indication bias, we performed two additional analyses: Stratification by year of therapy initiation (from 1996 to 1998 and after 1999, the year NNRTIs became available in Brazil); and, an analysis that included only the subgroup of patients with baseline CD4 cell counts less than 200/uL and who started therapy after 1999.

2.3.2 Study variables and outcomes

Demographic variables that were analyzed included: sex, age, risk behavior, race and education in years. HAART was defined as any combination of three or more antiretroviral drugs including two nucleoside reverse-transcriptase inhibitors (NRTI) and at least one protease inhibitor (PI) or, one nonnucleoside reverse-transcriptase inhibitor (NNRTI). Antiretroviral regimens were categorized into three major groups: PI (regimens containing two NRTI and one PI); NNRTI (regimens containing two NRTI and one NNRTI), ritonavir-boosted containing regimens (regimens containing two NRTI, low dose ritonavir, and at least one more PI). Patients started on regimens containing only NRTIs (two NRTI and abacavir) were excluded from the analysis because of their limited number.

Baseline CD4 counts and HIV plasma viral load were those measured up to 3 months before the start of therapy. Virological failure was defined as a HIV PVL greater than or equal to 400 copies/μL 3 to 9 months (closest to 6 months) after treatment initiation. When more than one measurement was available, the one closest in time to 6 months of therapy was selected. Non-adherence to therapy was self-reported, and was defined as having missed one or more doses in the week before the clinic visit. The 1993 revised CDC AIDS case definition was used [70].

2.3.3 Statistical analysis

An intent-to-continue-treatment approach, which ignored subsequent therapy changes or interruptions, was used. Univariate analyses were performed by using Chi Square or Fisher's exact test for categorical variables, and Student t test or Wilcoxon test for continuous variables. Relative risks or odds ratios and 95% CI were also determined. HIV plasma viral load and CD4 cell count were normalized using $\log_{10}$ and square root transformations, respectively. For multiple comparisons, we used the ANOVA procedure and comparisons between groups were adjusted for type I error using the Bonferroni correction. Variables with $p<0.10$ in the univariate analysis were included in the multivariate analyses. We used a stepwise approach to assess factors independently associated with outcome. All p values are two sided. SAS version 8.0 (SAS Institute, Cary, NC) was used for all analyses.

2.4 RESULTS

From 1996 to 2003, 1,976 patients started HAART, of whom 622 (31.5%) were antiretroviral naïve. Of these, 454 (73.0%) had information available at 6 months and were included in the study. One patient died before the sixth month of therapy and was therefore categorized as virological failure. Nearly one-half of the patients initiated HAART with a PI (208; 45.7%). The PI used was predominantly indinavir (92; 20.2%), followed by nelfinavir (81; 17.8%), saquinavir (27; 5.9%), and ritonavir (8; 1.7%). Around forty percent of the study subjects initiated HAART with a regimen that included a NNRTI. Among these, the vast majority received efavirenz (168; 36.9%), followed by nevirapine (11; 2.4%). Fifty-eight patients received a ritonavir boosted regimen (12.7%), 3 patients received a triple NRTI regimen, and 1 patient initiated a different regimen.

The following characteristics were similar for patients who were included in the study and for the 168 therapy naïve patients who were not eligible because a viral load measurement was not available 6 months after treatment initiation: age, gender, prior AIDS diagnosis, and baseline CD4 cell count and HIV PVL. However, those not included were 1.83 times (95% CI: 1.21–2.77) more likely to be non-adherent to treatment and 2.1 times (95% CI: 1.14–4.15) more likely to have received regimens with one PI, as compared to ritonavir-boosted regimens. They were also more likely to have started therapy before 1999, compared with those who started in 1999 and after (OR: 1.93; 95% CI: 1.35 –2.78). Sixty-five percent of included patients were men; the median age was 36 years, with 22.5% being less than 30 years old, 44.2% 31 to 40 years old, and 33.3% 41 years of age and older. The most common risk factors for HIV infection were heterosexual activity (43.9%) and being a man who reported having sex with men (35.7%). Baseline characteristics of the 454 patients are summarized in Table 3 to 5.

Table 3. Demographic and clinical baseline characteristics of 454 patients starting antiretroviral therapy from 1996 to 2004

Variable	**median (IQR), n/n (%)**
Median age (years)	36 (31–43)
Male	296/454 (65.2%)
White race	412/450 (91.6%)
Risk factor	
MSM	144/403 (35.7%)
IDU	77/403(19.1%)
Transfusion	5/403 (1.2%)
Heterosexual	177/403 (43.9%)
Less than 5 years of formal education	109/388 (28.1%)
CD4 count at baseline	

Table 3 continued:

Variable	median (IQR), n/n (%)
Median (cells/μL)	156 (47–253)
Count ≤100 cells/μL	159/420 (37.9%)
AIDS diagnosis	322/453 (71.1%)
HIV RNA level at baseline (log_{10} copies/mL)	5.0 (4.58–5.60)
Year therapy initiated	
1996 – 1997	76/454 (16.7%)
1998	69/454 (15.2%)
1999	84/454 (18.5%)
2000	58/454 (12.8%)
2001	63/454 (13.9%)
2002	55/454 (12.1%)
2003 – 2004	49/454 (10.8%)
Regimen type	
2 NRTI + 1 PI	208/454 (45.7%)
2 NRTI + 1 NNRTI	179/454 (39.5%)
RNV boosted	58/454 (12.8%)
NNRTI + PI	5/454 (1.1%)
3 NRTI	3/454 (.6%)
Other	1/454 (.2%)

MSM, men who have sex with men; IDU, injecting drug use; NRTI, nucleoside reverse-transcriptase inhibitor; NNRTI, non-nucleoside reverse-transcriptase inhibitor; PI, protease inhibitor; IQR, inter-quartile range.

Table 4. Demographics and baseline clinical characteristics according to initial treatment received.

	1 Protease inhibitor (n=207)	Non-nucleoside inhibitor (n=179)	Ritonavir boosted (n=58)	P
Sex male [n (%)]	145/207 (70.1%)	106/179 (59.2%)	39/58 (67.2%)	.21
Age (years) [median (IQR)]	34 (31–40)*	37 (31–44)	39 (33–49)*	.013

Table 4 continued:

	1 Protease inhibitor (n=207)	Non-nucleoside inhibitor (n=179)	Ritonavir boosted (n=58)	P
Non white ethnicity [n(%)]	16/207 (7.7%)	20/179 (11.2%)	5/58 (8.6%)	.51
Risk behavior [n(%)]				
MSM	79/187 (42.2%)	44/157 (28.0%)	19/50 (38.0%)	
IDU	36/187 (19.2%)	28/157 (17.8%)	12/50 (24.0%)	
Transfusion	4/187 (2.1%)	0	1/50 (2.0%)	
Heterosexual	68/187 (36.4%)	85/157 (54.1%)	18/50 (36.0%)	
Less than 5 years of education [n(%)]	43/176 (24.4%)	43/154 (28.1%)	19/51 (37.2%)	.083
AIDS diagnosis [n(%)]	146 (70.9%)	113 (63.1%)	53 (91.4%)	.092
Viral load at baseline (log_{10} copies/mL) [median (IQR)]	5.11 (4.72–5.65)*	4.80 (4.35–5.29)*	5.60 (5.22–6.04)*	<.0001
CD4 count at baseline (cells/mL) [median (IQR)]	132 (39–289)	203 (102–254)*	85 (37–159)*	.0029

*Pairwise comparisons with statistically significant difference

Table 5. Demographic and clinical baseline characteristics of patients stratified by year

Year	1996-97 (n=76)	1998 (n=69)	1999 (n=84)	2000 (n=58)	2001 (n=63)	2002 (n=55)	2003-04 (n=49)
Male	53 (69.7%)	52 (75.4%)*	55 (65.5%)	39 (67.2%)	39 (61.9%)	31 (56.4%)	27 (55.1%)
Age	33 (30–37)*	36 (32–44)	34 (31–42)	39 (32–46)*	38 (34–45)*	37 (29–42)	36 (32–46)
Non-white	3 (3.9%)	5 (7.3%)	13 (15.5%)	5 (8.6%)	5 (7.9%)	5 (9.1%)	6 (12.2%)
Risk behavior							
MSM	31/72 (43.1%)	30/56 (53.6%)	21/80 (26.2%)	22/48 (45.8%)	19/51 (37.2%)	11/49 (22.4%)	10/47 (21.3%)
IDU	9/72 (12.5%)	9/56 (16.0%)	19/80 (23.7%)	6/48 (12.5%)	7/51 (13.7%)	16/49 (32.6%)	11/47 (23.4%)
Transfusion	0	2/56 (3.6%)	2/80 (2.5%)	0	0	1/49 (2.0%)	0
Heterosexual	32/72 (44.4%)	15/56 (26.8%)	38/80 (47.5%)	20/48 (41.7%)	25/51 (49.0%)	21/49 (42.8%)	26/47 (55.3%)
Education Low vs High	8 (11.4%)*	14 (24.6%)	19 (27.9%)	19 (36.5%)	20 (36.4%)	16 (36.4%)	13 (30.9%)

Table 5 continued:

Year	1996-97 (n=76)	1998 (n=69)	1999 (n=84)	2000 (n=58)	2001 (n=63)	2002 (n=55)	2003-04 (n=49)
AIDS	49 (64.5%)	53 (76.8%)	57 (68.7%)	38 (65.5%)	52 (82.5%)*	43 (78.2%)	30 (61.2%)
Viral load at baseline	5.11 (4.74–5.67)	5.18 (4.79–5.61)	5.00 (4.50–5.62)	5.04 (4.57–5.63)	5.22 (4.64–5.74)	5.01 (4.37–5.47)	4.81 (4.36–5.23)
CD4 count at baseline	140 (34–330)	132 (40–222)	145 (48–289)	203 (41–254)	117 (40–214)	182 (71–244)	194 (122–230)

2.4.1 Outcomes at 6 months

After 3-9 months on HAART, 127 (27.9%) patients had detectable HIV plasma viral load. The median increase in CD4 count was 117 cells/uL and the median HIV PVL decrease was 2.99 $\log_{10}$ copies/mL. Table 6 summarizes the main outcomes at 6 months of therapy.

Table 6. Main outcomes at 6 months on HAART for 454 patients

Outcome	median (IQR), n/n (%)
Median PVL level at 6 months ($\log_{10}$ copies/mL)	1.90 (1.70–2.60)
Median decrease in HIV RNA($\log_{10}$ copies/mL)	2.99 (2.40–3.53)
Median CD4 count at 6 months (x 10^6 cells/L)	273 (174–415)
Median change in CD4 count (x 10^6 cells/L)	117 (60–197)
CD4 increase ≥50 x 10^6 cells/L	316/373 (84.7%)
CD4 increase ≥100 x 10^6 cells/L	236/373 (63.3%)
Virologic treatment failure	127/454 (27.9%)

In comparison with patients with virological success, those with virological failure were younger (median 34 vs. 37 years of age; $p<0.01$); had higher baseline HIV plasma viral load (5.34 vs. 5.00 $\log_{10}$ copies/uL; $p<0.01$) and lower baseline CD4 counts (86 vs. 182 cells/uL; $p<0.01$). Non-adherent patients were 1.39 times less likely to respond than adherent patients, as

well as patients who received regimens containing a single PI, as compared to those prescribed ritonavir-boosted regimens (OR: 8.58; 95% CI: 3.53–20.85) and NNRTI based regimens (OR: 0.73; 95% CI: 0.27–2.01). In the multivariate analysis, higher baseline HIV PVL was the only factor associated with virological failure (OR: 2.1; 95% CI: 1.1–4.0). The results are summarized in Table 7.

Table 7. Factors associated with virological failure among all 454 patients.

Categorical variables	**Univariate analysis**		
	RR	**95% CI**	**P**
Sex (Male)	1.10	0.98–1.23	0.12
Race Nonwhite vs White	0.91	0.76–1.07	0.32
Education			
Low vs high	0.94	0.82–1.08	0.43
Age group			
<30	2.51	1.39–4.52	**0.05**
30–40	2.36	1.41–3.96	**0.06**
>40 (ref)	1.00	–	**–**
Year			
1996 – 1997	125.6	16.29–969.42	**<0.01**
1998	66.19	8.63–507.51	**<0.01**
1999	8.78	1.11–69.38	0.75
2000	9.99	1.23–81.15	0.95
2001	2.39	0.24–23.80	**<0.01**
2002	4.79	0.54–42.58	0.11
2003 – 2004 (ref)	1.00	–	**–**
Lack of adherence	1.39	1.16–1.68	**<0.01**
AIDS	1.18	1.05–1.31	**<0.01**
Regimen			
2 NRTI + 1 PI	8.58	3.53–20.85	**<0.01**
2 NRTI + 1 NNRTI	0.73	0.27–2.01	**<0.01**
Reg. RNV boosted(ref)	1.00	–	–

Table 7 continued:

Continuous variables	**Virological failure median (IQR)**	**Viral suppression median (IQR)**	
Viral load at baseline	5.34 (4.90–5.83)	5.00 (4.50–5.52)	**<0.01**
CD4 count at baseline	86 (35–226)	182 (64–256)	**<0.01**

Type of HAART regimen was highly associated with the year in which therapy was started, with NNRTI-containing and ritonavir-boosted regimens becoming much more common over time. In addition, virologic failure was significantly higher for patients who started therapy before 1999. Among the 145 patients who started HAART between 1996 and 1998, the year before NNRTIs became available in Brazil, the virological failure rate was 65.5% versus 10.4% for the 309 patients who started therapy in 1999 or later ($p<0.01$).

Given these time-dependent trends and to provide information relevant to current treatment practices in Brazil, subsequent analyses were restricted to patients who began therapy in 1999 or later.

2.4.2 Sub-analysis of patients who started HAART in 1999 and later

In the univariate analysis of patients starting therapy after 1999, age < 30 years, previous AIDS-diagnosis, higher baseline plasma viral load, fewer than 5 years of formal education, non-adherence to therapy, and type of regimen used were associated with virologic failure. In the multivariate analysis, higher baseline plasma viral load (OR: 2.32; 95% CI: 1.03–5.25); and non-

adherence (OR: 4.77; 95% CI: 1.47–15.50) were independently associated with virological failure (Table 8).

Table 8. Factors associated with virological failure in the 309 patients who started HAART in 1999 and after.

Categorical variables	**Univariate analysis**			**Multivariate analysis**		
	RR	**95% CI**	**P**	**OR**	**95% CI**	**P**
Sex (Male)	1.03	0.95–1.11	0.39	–	–	–
Race Nonwhite vs White	1.01	0.89–1.15	0.77	–	–	–
<5years of education	1.08	0.98–1.19	0.06			
Lack of adherence	1.31	1.11–1.53	**<0.01**	4.77	1.47–15.50	**<0.01**
Age group						
<30	3.39	1.17–9.81	0.09			
30–40	2.74	1.03–7.26	0.29			
>40 (ref)	1.00	–	–			
AIDS	1.10	1.04–1.18	**0.02**			
Regimen						
2 NRTI + 1 PI	2.83*	0.86–9.35	**0.02**			
2 NRTI + 1 NNRTI	1.11	0.35–3.52	0.31			
RNV boosted(ref)	1.00	–	–			
Continuous variables	**Virological failure median (IQR)**	**Viral suppression median (IQR)**				
Viral load at baseline (log_{10} copies/mL)	5.60 (5.05–6.10)	4.98 (4.50–5.50)	**<0.01**	2.32	1.03–5.25	**0.04**
CD4 count at baseline (cells/μL)	104 (41–208)	175 (64–254)				

*OR

2.4.3 Sub-analysis of patients who started therapy in 1999 and after and had advanced disease

In the subset of 158 patients who started therapy after 1999 and whose baseline CD4 count was ≤200 cells/uL, factors independently associated with virological failure were non-adherence

(OR: 8.78; 95% CI: 1.49–51.80), and number of years of formal education (OR: 6.05 for those with < 5 years of formal education; 95%CI: 1.02–35.99). Results are summarized in Table 9.

Table 9. Factors associated with virological failure in 158 patients who started HAART in 1999 and after, and had CD4 count<200 cels/ uL.

Factor (categorical variables)	**Univariate analysis**			**Multivariate analysis**		
	RR	95% CI	P	OR	95% CI	P
Sex (Male)	0.98	0.88–1.10	0.81	–	–	–
Race Nonwhite vs White	1.01	0.84–1.21	0.90	–	–	–
Education						
Low vs high	1.17	1.01–1.36	**0.02**	6.05	1.02–35.99	**0.05**
Age group						
<30						
30–40						
>40 (ref)						
Lack of adherence	1.40	1.12–1.74	**<0.01**	8.78	1.49–51.80	**0.02**
Regimen						
2 NRTI + 1 PI	4.92	0.95–25.57	**0.02**			
2 NRTI + 1 NNRTI	1.73	0.34–8.71	.66			
Reg. RNV boosted (ref)	1.00	–	–			
Factor (continuous variables)	**Virological failure median (IQR)**	**Viral suppression median (IQR)**				
Viral load at baseline	5.73 (5.53–6.50)	5.11 (4.54–5.67)	**0.01**			
CD4 count at baseline	52 (32–103)	82 (27–134)	0.41			

2.5 DISCUSSION

There are limited data on the response to therapy in developing country settings [44]. In a meta-analysis of 10 observational studies conducted in developing countries, Ivers et al showed that ART resulted in an virological suppression in nearly 70% of individuals at time points up to

month 18 [57]. Since 1996, there has been free and universal access to antiretroviral therapy in Brazil to all individuals who qualify for treatment according to national guidelines. At present, over 150,000 Brazilians participate in this program. As a direct consequence, HIV-related morbidity and mortality have sharply declined [64]. Therapy guidelines are periodically revised by an independent advisory committee and, at present, Brazilian guidelines are similar to the IAS-USA guidelines [71]. Nonetheless, despite the enormous public health impact of the Brazilian program, there are few published reports on response to therapy [65-67].

In the present study, we investigated predictors of virological failure in therapy-naïve HIV-1 infected patients followed at a public hospital in southern Brazil after 3-9 months on HAART. We found that nearly three quarters of patients had undetectable viral load 3-9 months after starting therapy. When analyses were restricted to those starting treatment in 1999 and after, success rates were approximately 90% or more for all CD4 strata. Given that patients seen in the earlier years did not differ with regards to age, gender, risk behavior, prior AIDS diagnosis, and baseline HIV plasma viral load and CD4 cell count, the observed improvement in response rates over time is most likely due to the availability of more potent and easier to take regimens, such as those containing NNRTI and ritonavir-boosted regimens, and is in line with results from randomized controlled trials, and other observational studies that have demonstrated the superiority of these regimens over those containing a single PI [72, 73].

Similar to various other reports, adherence to therapy was associated with superior virological outcome [74-76]. Although it is possible that self-reported adherence may overestimate real adherence [77], several studies have demonstrated a correlation between self-reported adherence and electronic medication event monitoring systems [78-80]. Number of years of formal education was also found to be independently associated with virologic success

among patients with more advanced immunodeficiency. This finding is consistent with results from other studies conducted in Brazil and elsewhere. For example, Kalichman et al reported that the number of years of education and health literacy were predictors of treatment adherence in 318 patients taking antiretroviral therapy in a community sample [81]. In another study conducted in several public outpatient clinics in Rio de Janeiro, we demonstrated that lack of understanding about antiretroviral therapy was an important determinant of therapeutic failure [82].

This was a retrospective observational study that is subject to several limitations. First, patients for whom a viral load measurement was not available 3 to 9 months after treatment initiation were not included in the study. Since non-compliance with clinic appointments, a predictor of therapeutic success [11], is one of the likely reasons for the non-performance of monitoring laboratory tests, excluded patients are more likely to be non-adherent to treatment. Thus, it is possible that we have underestimated the overall risk of virological failure. Excluded patients were also more likely to have received single PI based regimens and have started therapy before 1999, which reflects clinical practices at that time. In fact, when these patients were considered as treatment failures, we found that lack of adherence (OR: 2.87; 95% CI: 1.88–4.37) and year of initiation before 1999 (OR: 6.58; 95% CI: 3.91–11.08) remained associated with virological failure, after adjusting for age, AIDS diagnosis, baseline CD4 count and viral load, and regimen. Given the observational nature of the study, the lack of randomization to different treatment regimens makes comparisons between the outcomes of different strategies susceptible to considerable selection-by-indication bias. We attempted to minimize the potential bias associated with changes in clinical practices over time by limiting some analyses to patients who started therapy in 1999 and after and to patients with more

advanced immune deficiency. Our results may also have been influenced by potentially confounding risk factors that were not included in the analysis [32, 72, 83]. The differences over time may reflect improvement in health care quality and provider's experience [84, 85]. We also may have been unable to accurately characterize virological success for some patients. For example, those patients for whom laboratory information was only available earlier may have been considered as treatment failures, as well as those who were slower responders. This study was of relatively short duration, which does not allow us to comment on the long-term success of HAART in our population. Further follow-up of the study patients is being carried out and will help provide insights on the role of virological response at 6 months on long-term outcomes, such as sustained response to therapy, the incidence of opportunistic infections, and death in this population. Finally, Brazil may be representative of middle-income developing countries but most likely is not representative of more resource-poor developing nations.

In summary, virologic response rates observed in a public clinic in Brazil compared favorably to what has been reported from developed countries settings as well as from developing countries. Our findings add to a growing body of literature that supports the effort of the World Health Organization to expand access to HAART in developing countries, where the vast majority of HIV infections occur. In this cohort, virologic success was associated with year of therapy initiation, consistent with the introduction of NNRTIs and ritonavir-boosted regimens into clinical practice. With currently available therapies, adherence and level of education were shown to be predictors of virological response, particularly in patients with more advanced immune deficiency. It was also shown that less than 5 years of formal education was associated with non-adherence, particularly among patients receiving regimens containing a single PI or ritonavir-boosted regimens (data not shown). These results suggest a need for interventions that

focus on improving adherence to HAART in persons with little formal education, particularly for those prescribed more complex regimens.

3.0 ARTICLE 3 – DISCORDANT RESPONSES TO POTENT ANTIRETROVIRAL TREATMENT IN PREVIOUSLY NAÏVE HIV-1 INFECTED ADULTS INITIATING TREATMENT IN RESOURCE-CONSTRAINED COUNTRIES

The Antiretroviral Therapy in Low Income Countries (ART-LINC) Collaboration*

(From manuscript originally published in the Journal of Acquired Immune Deficiency Syndrome 45(1): 52-9, 2007)

*Members of the study groups who made this collaboration possible are listed at end of paper (see Acknowlegments on page 53)

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Running Head: Discordant responses to antiretroviral treatment in resource-constrained settings

3.1 ABSTRACT

Objectives: To assess frequency of and risk factors for discordant responses at 6 months on HAART in previously treatment-naïve HIV-patients from resource-limited countries.

Methods: The Antiretroviral Therapy in Lower Income Countries (ART-LINC) Collaboration is a network of clinics providing care and treatment to HIV infected patients in Africa, Latin America, and Asia. Patients who initiated therapy between 1996 and 2004, were aged 16 years or older, and had a baseline CD4 cell count were included in this analysis. Responses were defined based on plasma viral load and CD4 cell count at 6 month as complete ($VR^{+}IR^{+}$); virologic only ($VR^{+}IR^{-}$); immunologic only ($VR^{-}IR^{+}$) and non-response ($VR^{-}IR^{-}$). Multinomial logistic regression was used to assess the association between therapy responses and clinical and demographic variables.

Results: Of the 3,111 patients eligible for analysis, 1,914 had available information at 6 months of therapy: 1,074(56.1%) were $VR^{+}IR^{+}$, 364(19.0%) were $VR^{+}IR^{-}$, 283(14.8%) were ($VR^{-}IR^{+}$), 193(10.1%) were $VR^{-}IR^{-}$. Compared to complete responders, virologic only responders were older, had higher baseline CD4 counts, lower baseline viral load, and were more likely to have received a nonstandard HAART regimen; immunologic only responders were younger, had a lower baseline CD4 count, a higher baseline viral load and were more likely to have received a PI based regimen.

Conclusions: The frequency of and risk factors for discordant response were comparable to those observed in developed countries. A longer follow-up is needed to assess the long-term impact of discordant responses on mortality in these resource-limited settings.

3.2 INTRODUCTION

In high income countries, the availability of highly active antiretroviral therapy (HAART) has led to major reductions in morbidity and mortality from HIV infection [1-3]. Several studies in this setting have demonstrated a stronger correlation between long-term prognosis and virologic and immunologic responses after 6 months on HAART than with baseline values [18, 86, 87]. In general, the initiation of HAART leads to a significant reduction in plasma HIV plasma viral load (PVL) and an increase in CD4 cell counts [7, 12]. However, some patients on HAART exhibit a pattern of sustained CD4 response despite persistent viremia, or do not exhibit a significant rise in CD4 cell counts despite viral suppression. Both situations are referred to as discordant responses and have been consistently associated with an intermediate risk of developing an AIDS event or death in developed countries [15, 20, 26, 88].

In high-income countries, discordant responses have been reported to occur in 20% to 30% of patients 6 months to 2 years after starting HAART [15, 19, 24, 86, 89, 90]. In developing countries, the effectiveness of HAART in suppressing viral replication and inducing CD4 cell rise is comparable to what has been reported from developed countries [56, 57, 82, 91-93]. Nonetheless, to date there is scant information on the frequency and prognostic significance of discordant responses to HAART in low-income countries, where patients often start therapy at advanced stages of immune deficiency and frequently have co-morbidities that may impair response to therapy [94].

We report on the frequency of and risk factors for discordant responses at 6 months on HAART in previously treatment naïve HIV-patients in resource-limited countries.

3.3 METHODS

3.3.1 Patients and measurements

The Antiretroviral Therapy in Lower Income Countries (ART-LINC) Collaboration is a network of HIV treatment programmes in Africa, Latin America, and Asia. The ART-LINC collaboration has been described previously [58, 62]. The selection of patients and data extraction were done at the participating sites. Data were anonymized locally, then pooled and analysed centrally. At all sites local ethics committees or institutional review boards approved the collection of data.

Only sites that collected HIV PVL routinely were included in the analysis. All previously treatment-naïve individuals who initiated therapy between March 1996 and April 2004, had a known date of therapy initiation, were aged 16 years or older, and had a documented CD4 cell count at baseline were included in this analysis.

Type of HAART regimen was defined as protease inhibitor (PI)-based (one PI plus two nucleoside reverse-transcriptase inhibitors [NRTI], including ritonavir-boosted regimens), non-nucleoside reverse transcriptase (NNRTI)-based (one NNRTI plus two NRTIs), or a nonstandard HAART regimen (including triple NRTI regimens and any other regimen containing a minimum of 3 drugs). Stage of disease was classified as less (CDC stage A/B, WHO stages I or II) or more advanced (CDC stage C, WHO stages III or IV). Baseline CD4 cell count and HIV plasma viral load (PVL) were measured at therapy initiation (-180 or + 7 days).

3.3.2 Study Outcomes and Statistical Analysis

Virologic response (VR) was defined as achieving a plasma HIV PVL < 500 copies/mL, whereas immunologic response (IR) was defined as an increase of at least 50 CD4 cells/μL at 6 months. Measurements closest to 6 months after starting HAART, within 3 to 9 months, were used in

these analyses. Outcomes were defined as complete response ($VR^{+}IR^{+}$); virologic only response ($VR^{+}IR^{-}$); immunologic only response ($VR^{-}IR^{+}$) and non-response ($VR^{-}IR^{-}$). An intent-to-continue-treatment approach, which ignored subsequent therapy changes or interruptions, was used. Between-group comparisons were made by using Chi-Square test for categorical variables and Kruskall-Wallis test for continuous variables. A multinomial logistic regression model was fit to assess the relationship between baseline characteristics and the 6 month outcomes. Heterogeneity introduced by different sites was accounted for by including site as a fixed effect in the model, and Huber-White robust standard errors were calculated to account for intra-site correlation.

Missing baseline information on stage of disease and PVL were multiply imputed, based on whether the patient died, site, CD4 cell count, sex, age and type of HAART regime. In these imputations, values of the missing data were randomly sampled from their predicted distributions. Analyses were run on each of 20 datasets that included the imputed values, and the results combined with Rubin's rules [95]. Analyses were performed using Stata version 9.0 (Stata Corp., College Station, TX).

3.4 RESULTS

During the study period, 4,810 patients initiated HAART. Of these, 158 (3.2%) died within 6 months of therapy, and 1,541 (32.0%) were from sites where viral load was not routinely performed and therefore were not included in the analysis. The demographic and clinical characteristics of the remaining 3,111 patients are shown in Table 10 according to immunologic and virologic responses at 6 months on therapy.

Table 10. Patients' baseline characteristics according to immunologic and virologic response at 6 months of therapy and comparison between those with known and unknown response.

Variable	Total N = 3111	VR+IR+ N = 1074	VR+IR- N = 364	VR-IR+ N = 283	VR-IR- N = 193	Unknown Response N = 1197	Known vs Unknown P
Male gender n (%)	1,585 (50.9)	532 (49.5)	195 (53.6)	144 (50.9)	97 (50.3)	617 (51.6)	0.43
Age years*	35 (30 – 41)	35 (30 – 41)	36 (31 – 42)	35 (28 – 41)	37 (31 – 42)	35 (30 – 42)	
Age groups n (%)							0.36
16 – 29	700 (22.5)	256 (23.8)	69 (18.9)	85 (30.0)	36 (18.6)	254 (21.2)	
30 – 39	1,435 (46.1)	507 (47.2)	172 (47.2)	119 (42.0)	86 (44.6)	551 (46.0)	
40 – 49	705 (22.9)	232 (21.6)	83 (22.8)	53 (18.7)	57 (29.5)	290 (24.2)	
≥ 50	261 (8.4)	79 (7.4)	40 (10.9)	26 (9.2)	14 (7.2)	102 (8.5)	
Baseline CD4 count cells/μL*	137 (49 – 240)	135 (49 – 240)	186 (87 – 282)	103 (36 – 207)	141 (50 – 263)	127 (45 – 225)	
Baseline CD4 count cells/μL at baseline n (%)							0.29
<50	782 (25.1)	272 (25.3)	51 (14.0)	91 (32.2)	47 (24.3)	321 (26.8)	
50 – 99	466 (14.9)	153 (14.2)	48 (13.2)	49 (17.3)	26 (13.5)	190 (15.8)	
100 – 199	790 (25.4)	270 (25.1)	97 (26.6)	68 (24.0)	44 (22.8)	311 (25.9)	
200 – 349	764 (24.6)	276 (25.7)	118 (32.4)	55 (19.4)	44 (22.8)	271 (22.6)	
≥ 350	309 (9.9)	103 (9.6)	50 (13.7)	20 (7.1)	32 (16.6)	104 (8.7)	
Baseline HIV RNA log_{10} copies/mL*	5.1 (4.6–5.6)	5.0 (4.6 – 5.6)	4.8 (4.4 – 5.4)	5.4 (4.8 – 5.8)	5.3 (4.8 – 5.7)	5.1 (4.6 – 5.6)	
Baseline HIV RNA copies/mL* - n (%)							<0.01
< 10,000	194 (6.2)	78 (7.3)	43 (11.8)	15 (5.3)	12 (6.2)	46 (3.8)	
10000 – 99999	747 (24.0)	350 (32.6)	126 (34.6)	51 (18.0)	46 (23.8)	174 (14.5)	
≥ 100000	1,181 (37.9)	484 (45.1)	138 (37.9)	146 (51.6)	107 (55.4)	306 (25.5)	
Unknown	989 (31.8)	162 (15.6)	57 (15.6)	71 (25.1)	28 (14.5)	671 (56.1)	
Clinical stage							<0.01
Less advanced	778 (25.0)	243 (22.6)	111 (30.5)	66 (23.3)	53 (27.5)	305 (25.5)	
More advanced	1,591 (51.1)	594 (55.3)	179 (49.2)	158 (55.8)	103 (53.4)	557 (46.5)	
Unknown	742 (23.8)	237 (22.1)	74 (20.3)	59 (20.8)	37 (19.2)	335 (27.9)	
Regimen type							<0.01

Table 10 continued:

Variable	Total N = 3111	VR+IR+ N = 1074	VR+IR- N = 364	VR-IR+ N = 283	VR-IR- N = 193	Unknown Response N = 1197	Known vs Unknown P
NNRTI based	1,783 (57.3)	658 (61.3)	202 (55.5)	96 (33.9)	68 (35.2)	759 (63.4)	
PI based	889 (28.6)	289 (26.9)	94 (25.8)	125 (44.2)	54 (27.9)	327 (27.3)	
Other	439 (14.1)	127 (11.8)	68 (18.7)	62 (21.9)	71 (36.8)	111 (9.3)	
Calendar year							<.01
1996-1997	178 (5.7)	58 (5.4)	17 (4.7)	25 (8.8)	16 (8.3)	62 (5.2)	
1998	285 (9.2)	73 (6.8)	35 (9.6)	55 (19.4)	39 (20.2)	83 (6.9)	
1999	430 (13.8)	148 (13.8)	67 (18.4)	68 (24.0)	52 (26.9)	95 (7.9)	
2000	487 (15.6)	194 (18.1)	62 (17.0)	53 (18.7)	28 (14.5)	150 (12.5)	
2001	556 (17.9)	211 (19.6)	67 (18.4)	38 (13.4)	23 (11.9)	217 (18.1)	
2002	674 (21.7)	253 (23.6)	79 (21.7)	31 (10.9)	26 (13.5)	285 (23.8)	
2003-2004	501 (16.1)	137 (12.8)	37 (10.2)	13 (4.6)	9 (4.6)	305 (25.5)	

* Median (IQR)

About half were male (50.9%), with a median age at HAART initiation of 35 years. The median baseline CD4 cell count and HIV-RNA plasma viral load were 137 (IQR 49 – 240) cells/μL and 5.1 (IQR 4.6 – 5.6) $\log_{10}$ copies/mL respectively, 1,591(51.1%) had more advanced disease, and 1,783 (57.3%) were prescribed a NNRTI based regimen. Excluded patients were more likely to be female (55% vs 45%, $p<0.01$) and to have a lower baseline CD4 cell count (70 vs 136, $p<0.01$).

The majority of patients (57.3%) started therapy with a NNRTI based regimen, and 29 % started with a PI based regimen. The most prescribed NNRTI and PI were efavirenz (66.1%) and indinavir (48.8%), respectivelly. PI based regimens were more commonly prescribed before the year of 2000 (64.0%). From 2000 onwards, NNRTI based regimens predominated (76.0%).

Recipients of nonstandard HAART regimens had the highest median baseline CD4 count – 185 (IQR 63 – 315) cells/μL, whereas recipients of PI based and NNRTI based regimens had similar median baseline counts: 131 (IQR 45 – 253) and 131 (IQR 48 – 222) cells/μL, respectively. The baseline viral load for recipients of NNRTI, PI based and non-standard regimens were 5.1 (IQR 4.6 – 5.6), 5.2 (IQR 4.7 – 5.7), and 4.9 (IQR 4.5 – 5.5) $\log_{10}$ copies/mL, respectively (data not shown).

3.4.1 Immunologic and virologic responses at 6 months of therapy

At 6 months on therapy, 1,914 (61.5%) patients had information on PVL and CD4 cell count. For 1,197 (38.5%), the outcome could not be ascertained because of missing data on either PVL or CD4 count. Among these patients, 292 (24.4%) were lost to follow up at 6 months.

Among the patients with available information, 1,074 (56.1%) were complete responders, 364 (19.0%) were virologic only responders, 283 (14.8%) were immunologic only responders, and 193 (10.1%) were nonresponders (Table 11). The overall virologic response rate was 75.1%, the median increase in CD4 count was +106 (IQR +40 – +178) cells/μL, and the median viral load reduction was 2.8 (IQR -3.4 – -1.9) $\log_{10}$copies/mL. Complete responders showed the greatest CD4 cell count increase and PVL decrease, and non-responders showed the smallest changes in these measurements.

Table 11. Outcomes at 6 months of therapy for 1914 patients with known outcome

Outcome (n)	Total	VR+IR+	VR+IR-	VR-IR+	VR-IR-
Virologic and immunologic response n (%)		1 074 (56.1)	364 (19.07)	283 (14.8)	193 (10.1)
CD4 count change (median (IQR) cells/mm^3)	+106 (40 – 178)	+148 (+102 – +225)	+12 (-14 – +33)	+125 (+85 - +194)	-1 (-42 – +16)
Viral load change ($\log_{10}$ (IQR) copies/mL)	-2.8 (-3.4 – -1.9)	-3.1 (-3.6 – -2.5)	-2.9 (-3.4 – -2.2)	-1.6 (-2.2 – -0.8)	+0.7 (-1.7 – 0)

Table 12 shows the results of the multinomial logistic regression model for 1,914 patients with known response, with complete responders as the reference group, controlling for demographic and clinical variables, calendar year, and participating site. There were no significant differences by gender between complete responders and all other categories of response.

Table 12. Odds Ratios (OR) and 95% confidence intervals (CIs) of discordant responses relative to complete response in 1,914 patients with known outcome.*

Variable	VR^+IR^- OR (95%CI)	P	VR^-IR^+ OR (95%CI)	P	VR^-IR^- OR (95%CI)	P	VR^+IR^- vs VR^-IR^+ P
Gender							0.13
Male	1.0		1.0		1.0		
Female	0.85 (0.70 – 1.03)	0.10	1.03 (0.73 – 1.45)	0.86	0.91 (0.61 – 1.36)	0.65	
Age groups							<0.01
16 – 29	1.0		1.0		1.0		
30 – 39	1.29 (0.94 – 1.78)	0.11	0.56 (0.42 – 0.75)	<0.01	0.88 (0.57 – 1.37)	0.59	
40 – 49	1.37 (0.87 – 2.16)	0.17	0.47 (0.29 – 0.77)	<0.01	1.31 (0.92 – 1.87)	0.13	
≥ 50	2.12 (1.45 – 3.09)	<0.01	0.64 (0.43 –0.95)	0.03	1.16 (0.78 – 1.73)	0.46	
Baseline CD4 count (cells/μL)							<0.01
<50	1.0		1.0		1.0		
50 – 99	1.70 (0.95 – 3.04)	0.07	0.96 (0.64 – 1.43)	0.85	1.09 (0.59 – 2.02)	0.77	
100 – 199	1.85 (1.43 – 2.40)	<0.01	0.73 (0.54 – 0.98)	0.04	1.04 (0.66 – 1.63)	0.86	
200 – 349	2.27 (1.71 – 3.03)	<0.01	0.58 (0.30 – 1.13)	0.11	1.18 (0.66 – 2.11)	0.57	
≥ 350	2.18 (1.47 – 3.23)	<0.01	0.34 (0.18 – 0.63)	<0.01	1.43 (0.60 – 3.39)	0.41	
Baseline HIV RNA copies/mL**							<0.01
< 10,000	1.0		1.0		1.0		
10,000 – 99,999	0.74 (0.45 – 1.22)	0.24	0.97 (0.53 – 1.77)	0.93	1.16 (0.45 – 2.94)	0.75	

Table 12 continued:

Variable	VR^+IR^- OR (95%CI)	P	VR^-IR^+ OR (95%CI)	P	VR^-IR^- OR (95%CI)	P	VR^+IR^- vs VR^-IR^+ P
≥ 100,000	0.63 (0.42 – 0.95)	0.03	1.77 (1.04 – 3.00)	0.03	1.81 (0.79 – 4.14)	0.16	
Clinical stage***							0.52
Less advanced	1.0		1.0		1.0		
More advanced	1.09 (0.77 – 1.54)	0.60	1.09 (0.73 – 1.64)	0.64	1.48 (0.87 – 2.51)	0.14	
Regimen type							<0.01
NNRTI based	1.0		1.0		1.0		
PI based	0.86 (0.74 – 1.01)	0.07	1.58 (1.08 – 2.30)	0.02	0.95 (0.48 – 1.85)	0.87	
Other	1.45 (1.00 – 2.11)	0.05	1.25 (0.86 – 1.81)	0.23	1.10 (0.56 – 2.18)	0.76	
Calendar year							<0.01
1996-1997	1.0		1.0		1.0		
1998	1.31 (0.62 – 2.76)	0.47	1.28 (0.46 – 3.53)	0.63	1.19 (0.50 – 2.81)	0.68	
1999	1.25 (0.86 – 1.81)	0.23	0.70 (0.30 – 1.63)	0.41	0.72 (0.40 – 1.27)	0.26	
2000	0.99 (0.60 – 1.64)	0.98	0.36 (0.13 – 0.97)	0.04	0.26 (0.09 – 0.69)	<0.01	
2001	0.99 (0.60 – 1.64)	0.97	0.29 (0.13 – 0.66)	<0.01	0.31 (0.13 – 0.72)	<0.01	
2002	1.09 (0.68 – 1.73)	0.72	0.31 (0.13 – 0.71)	<0.01	0.41 (0.14 – 1.19)	0.10	
2003-2004	0.88 (0.48 – 1.60)	0.69	0.22 (0.13 – 0.35)	<0.01	0.26 (0.07 – 0.94)	0.04	

*Odds ratios adjusted for site and year of therapy initiation as fixed effects.

**Baseline values imputed for 318 patients

***Baseline values imputed for 407 patients

In comparison with complete responders, virologic only responders were significantly more likely to be aged over 50, to have a baseline CD4 count >99 cells/μL and/or to have received nonstandard HAART regimens, and less likely to have baseline HIV RNA plasma viral load > 100,000 copies/mL.

Patients who showed an immunologic only response were less likely than complete responders to be older than 30 years, to have a baseline CD4 > 99 cells/ μL, and more likely to

have a baseline HIV RNA plasma viral load >100,000 copies/mL, or have received a PI-based regimen (Table 12). The probability of having an immunologic only or nonresponse rather than a complete response decreased after the year of 2000 (data not shown).

Some covariates had opposite effects on the two categories of discordant responses. Increasing age, increasing baseline CD4 count, and other regimens were positively associated with virologic only responses and PI based regimens were positively associated with immunologic only responses, while ≥ 100,000 baseline HIV RNA copies/mL was negatively associated with virologic only responses and positively associated with immunologic only responses. Figure 1 shows the adjusted odds ratios of discordant responses by baseline CD4 cell count strata with complete responders as the reference group.

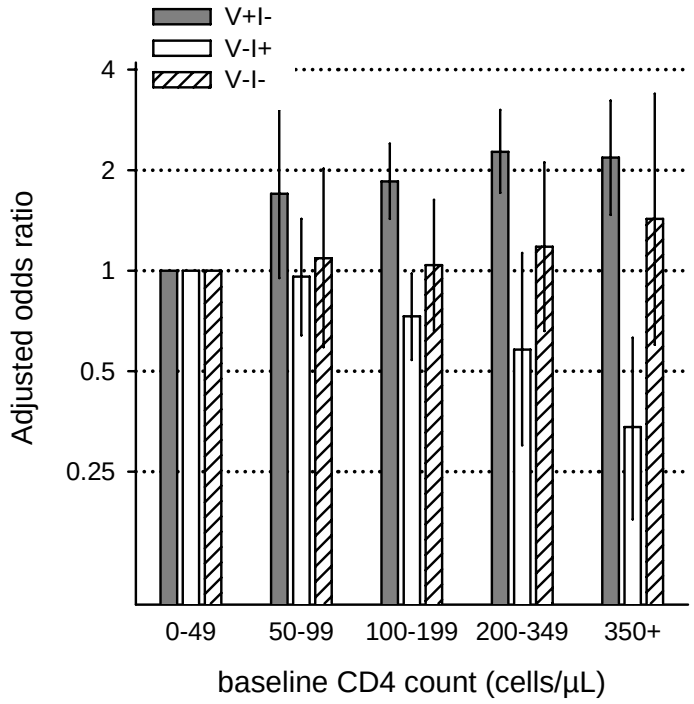

Figure 1. Adjusted odds ratios of responses (in log scale) after 6 months of HAART relative to complete response by baseline CD4 cell count**.**

We assessed the extent to which the rate of unknown response could have introduced bias in our analysis by comparing baseline characteristics, as well as virologic and immunologic responses between patients with known and unknown responses at 6 months. Patients with known and unknown responses at 6 months did not differ with respect to gender, age, or baseline CD4 cell count (Table 10). Patients with unknown response were more likely to have received a NNRTI based regimen, and to have an unknown baseline PVL and stage of disease. At 6 months of therapy, however, patients with unknown response and who had available information on either virologic or immunologic response (but not both) had overall similar median changes in CD4 cell count and PVL than did patients with both responses known (+112 vs +105, p = 0.26; and -2.7 vs -2.8, p = 0.88, respectively). A multinomial model including a missing outcome category led to similar results, supporting the hypothesis that this subgroup of patients did not significantly differ from the 1,914 patients with known outcome.

3.5 DISCUSSION

To our knowledge, this is the first report on the frequency of and risk factors for discordant responses in a large cohort of patients initiating HAART in low-income countries. The encountered frequency of discordant response (33.8%) at 6 months of therapy is similar to what has been reported from high-income countries[19, 20, 24, 88, 90]. We have found that, compared to complete responders, virologic only responders were older, had higher baseline CD4 counts, lower baseline viral load, and were more likely to have received a nonstandard HAART regimen; and immunologic only responders were younger, had a lower baseline CD4 count, and were more likely to have received a PI based or a nonstandard regimen.

Our finding of older age being associated with virologic only response and inversely associated with immunologic only response is consistent with studies conducted in high-income

countries [20, 25, 26, 88], as well as the hypothesis that the magnitude of immune restoration is dependent on thymic activity, which decreases with age [27]. Other reports from high-income countries also have demonstrated that older age is independently associated with impaired immunologic responses despite sustained virologic responses [44]. It is also suggestive of a better adherence among older patients, in agreement with other studies [96, 97].

As in reports from high-income countries, a higher baseline CD4 count was associated with an increased probability of a virologic only response and with a reduced probability of an immunologic only response [20, 88, 98]. One possible explanation for this finding is the non-linear nature of CD4 cell increase after HAART initiation across the different baseline CD4 count strata. In agreement with the findings of Moore et al.[88], our results also suggest that increases in CD4 counts following initiation of therapy might be greater in individuals with lower CD4 counts at therapy initiation. A baseline viral load above 100,000 copies/mL was associated with a lower probability of having a virologic only response, a finding also reported in the studies of Moore et al. and Nicastri et al.[20].

Our analysis showed that immunologic only responders were 1.6 times more likely than complete responders to have received PI-based regimens rather than NNRTI based regimens. This finding has to be interpreted with caution, however. As noted before, differences in response between HAART regimens in this observational study are likely to be subject to selection-by-indication bias. Nonetheless, it has been suggested that the additional effect of PIs on immunologic response could be due to their ability to reduce T-cell apoptosis [89]. A superior immunologic response to PIs over NNRTIs was suggested among virologic responders [99], but not among patients showing discordant responses [25, 88, 100]. This issue is unlikely to be fully elucidated by observational studies.

Qualitative differences in the effects of predictors of the two types of discordant responses support the hypothesis of different underlying mechanisms. Although the long term clinical outcomes seem to be comparable [88], identifying risk factors for both types of early discordant response may lead to specific preventive strategies for each type.

Discordant responses have been associated with increased risk of clinical progression and mortality in developed countries. In one cohort of antiretroviral experienced patients with advanced HIV disease who started PI based HAART and were followed for over 30 months, discordant responders at 12 months experienced significantly more AIDS-defining events than full responders, with immunologic only responders presenting a slightly higher probability of being event-free compared to virologic only responders [25]. In another study involving over 2,100 antiretroviral experienced and naïve HIV patients followed for a median of 44 months, immunologic only and virologic only responders had a significantly lower risk of clinical progression than non-responders, but a 2.3 and 1.9-fold greater risk of death or new AIDS-defining event than complete responders, respectively [20].

Little is known about the mechanisms underlying the development of discordant responses, but it apparently is dependent on the interaction of a multitude of viral and host factors. One hypothesis is that HAART selects viral strains that are less fit, which, in turn, results in reduced pathogenicity of drug resistant viruses. In fact, it has been shown that recipients of PI based regimens with prolonged discordance (immunologic success despite virological failure) have decreased viral replication capacity [45, 46, 51]. In one closely followed cohort of HAART naïve patients for whom repeated measures over a period of 1 year were analyzed, almost all patients who showed discordant immunologic response at 1 year had had either a prior transient period of undetectable plasma viral load and/or partial suppression of viral load to <1,000 copies/mL,

suggesting that partial viral suppression is the primary mechanism involved in discordant CD4 cell count increases [44]. Genetic variability, such as polymorphisms associated with drug transportation [32] and T lymphocyte apoptosis [38, 39] has been implicated in the pathogenesis of virologic only response. In addition, the concomitant use of tenofovir and didanosine has been shown to cause impaired immunologic response [43, 101]. In ART-LINC the negligible proportion of patients initiating therapy with this combination is not likely to have influenced our results.

A major strength of the present study is the large number of previously naïve patients starting therapy with NNRTI-based regimens. Most studies published so far have been conducted in developed countries and have included experienced patients receiving PI based regimens; these patients are not representative of patients starting therapy in resource limited settings, where the majority start treatment with NNRTI based regimens [58, 62].

Our study has several limitations. First, this study did not address the impact of adherence on outcomes. In the study of Moore et al.[88], suboptimal adherence was predictive of both virologic and immunologic only responses rather than complete response. Second, additional variability may have been introduced due to differences in population genetics or infecting HIV subtypes, which were not considered in the present analysis. Third, patients prescribed NNRTI had lower plasma HIV-RNA levels and higher CD4 cell counts than patients prescribed PIs, highlighting the importance of provider bias determining differences between different regimens [102]. Four, we acknowledge that the 38% unknown response rate potentially could affect our results. However, our analysis showed that this group of patients did not differ significantly from the patients in the other categories of response with respect to major baseline risk factors. We believe that the availability of laboratory resources on site or other factors that limited access to

laboratory tests were determinants of this response pattern rather than patient characteristics. In addition, including patients with unknown response at 6 months did not substantially change the estimates in the final model. Finally, if the limited availability of resources caused programmes to prioritize monitoring virologic and immunologic responses in patients who did not appear to be doing well clinically, we could have underestimated the virologic and the immunologic effectiveness of HAART in these settings.

Our results add to accumulating data on response to therapy in resource-limited countries, and may have important public health implications. We showed that despite considerable differences in disease severity at presentation and baseline CD4 count patients in resource-limited settings, the frequency of and risk factors for discordant response are similar to those observed in developed countries. In these countries, higher mortality increasingly has been reported for discordant responders than for complete responders. Clinical management of these patients often requires both a more sophisticated laboratory approach (genotypic and phenotypic resistance) and the availability of a second line therapy. Data on the frequency of this phenomenon and identification of its risk factors are of capital importance and may help HIV/AIDS programmes plan their laboratory and therapeutic resources. Further studies are needed to assess the long-term impact of early discordant responses in these resource-limited countries.

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4.0 ARTICLE 4 – EARLY IMMUNOLOGIC AND VIROLOGIC DISCORDANT RESPONSES AND MORTALITY IN PREVIOUSLY THERAPY-NAÏVE HIV-1 INFECTED ADULTS INITIATING TREATMENT IN RESOURCE-CONSTRAINED COUNTRIES

4.1 ABSTRACT

Objectives: We assessed the impact of immunologic and virologic patterns of response at 6 months of Highly Active Antiretroviral Therapy (HAART) in HIV-infected individuals from resource-limited countries.

Methods: All previously naïve participants who initiated HAART between 1996 and 2007, were aged 16 years or older, had a known date of therapy initiation, and a documented baseline CD4 were eligible for inclusion. Six (3 to 9) month response to therapy was categorized according to virologic, and immunologic responses considered jointly in: complete, virologic only, immunologic only; absent; and unknown response. Association between independent variables and outcomes were assessed by Cox proportional hazards regression. Robust standard errors were calculated to account for intra-site correlation.

Results: A total of 11,380 patients were included in the analysis, and 386 deaths were reported during 19,295 person-years (PY), corresponding to a mortality rate of 2.00 (95% CI 1.81 – 2.21) per 100 PY. In multivariable analysis adjusted for age at HAART initiation, clinical stage, baseline CD4 cell count, history of an AIDS defining condition after the 6 month response, cohort, and year of HAART initiation, discordant immunologic and virologic responses and non-responses were associated with increased risk of death. Compared with complete responders, virologic-only responders had a hazard of 2.29 (95% CI 1.64 – 3.20), immunologic-only responders had a HR of 2.22 (95% CI 1.52 – 3.26), and non-responders had a HR of 6.21 (95% CI 4.23 – 9.13).

Conclusions: Similar to what has been reported from developed countries, discordant immunologic and virologic responses were associated with intermediate risk of death in this large cohort of HIV-1 patients from resource-limited countries.

4.2 INTRODUCTION

The initiation of HAART generally leads to a rapid reduction in HIV-1 RNA plasma levels and to an increase in peripheral CD4+ cell counts [5, 7, 8]. However, some patients experience a discordant response, whereby the HIV-1 RNA plasma level is below the limit of detection but the CD4+ cell count increase is blunted. Other patients exhibit a different pattern of discordant response, characterized by a sustained CD4+ cell count increase, despite persistent viremia. Published data have indicated that, in comparison to complete response, discordant responses are associated with an intermediate risk of death or clinical progression[20, 22, 25, 87].

In the study of Grabar et al., virologic only responders and non-responders had a higher probability of clinical progression, whereas immunologic only responders and complete responders had similar risks [15]. In contrast, other studies have shown that immunologic only response also was associated with a higher risk of clinical progression. In one cohort of antiretroviral experienced patients with advanced HIV disease starting PI based HAART, that was followed for over 30 months, discordant responders at 12 months experienced significantly more AIDS-defining events than complete responders, with immunologic only responders having a slightly higher probability of being event-free compared to virologic only responders [25]. In another study involving over 2,100 antiretroviral experienced and naïve HIV patients followed for a median of 44 months, compared to complete responders, immunologic only and virologic only responders had significantly lower risk of clinical progression than non-responders, but a 2.3 and 1.9-fold greater risks of death or of experiencing a new AIDS-defining event, respectively [20].

Very few studies have assessed the prognostic value of discordant responses in naïve patients and in recipients of NNRTI-base regimens. In the study of Moore et al., mortality was

increased in patients showing an early discordant response, but no statistically significant difference was found between immunologic and virologic only responders [22]. Likewise, in a study involving HAART naïve IDU patients, discordant responders had an increased mortality compared to complete responders, but progression rates did not differ by whether early response was immunologic only or virologic only [90]. No published study has assessed the prognostic value of discordant responses in low-income countries. Our aim was to assess the association between immunovirologic discordant responses at 6 months of therapy and mortality in the ART-LINC collaboration.

4.3 METHODS

4.3.1 Study population

The Antiretroviral in Lower Income Countries collaboration of the International Databases to Evaluate AIDS (ART-LINC of IeDEA) is a large collaborative network of HIV/AIDS treatment programmes in low and middle income countries in Africa, South America and Asia. The collaboration has been described in detail elsewhere [58, 62]. In brief, the collaboration was established in 2003 with the aim of characterizing the prognosis of HIV-infected patients treated with HAART in resource-limited settings, to compare the experience between different settings, delivery modes and types of monitoring; and to compare outcomes with those observed in industrialized nations. The data collected at participating sites are transferred to data management and statistical teams at the universities of Bern, Switzerland and Bordeaux, France where data are cleaned, merged and analyzed according to established protocols. The present analysis includes all data available up to 29 June 2007. All previously naïve participants in ART-LINC who initiated HAART between 1996 and 2007, who were aged 16 years or older at

treatment initiation, had a known date of therapy initiation, and a documented baseline CD4 were eligible for inclusion. Only sites that collected HIV PVL routinely were included in the analysis. Patients who were missing both PVL and CD4 cell count at 6 (3 – 9) months were excluded. At all sites, local ethics committees or institutional review boards approved the collection of data.

The following independent variables were assessed: age at therapy initiation (years); gender (female/male); site; stage of disease at HAART initiation, classified as less (CDC stage A/B, WHO stages I or II) or more advanced (CDC stage C, WHO stages III or IV); incidence of an opportunistic infection; baseline CD4 cell count (cells/μL); 6 (3 to 9) month response to therapy, according to virological (HIV PVL < 500 copies/mL), and immunological (increase of at least 50 CD4 cells/μL) responses considered jointly, i.e., complete (VR+IR+), virologic only (VR+IR-), immunologic only (VR-IR+); absent (VR-IR-); and unknown response. First-line HAART regimens were categorized as NNRTI based (one NNRTI plus two NRTIs); PI based (one PI plus two NRTIs or ritonavir-boosted regimens); or other (including triple NRTI regimens and any other regimen containing a minimum of 3 drugs). Sites were categorized according to regions into Africa, Asia, and South America.

4.3.2 Outcomes

The endpoint was all cause mortality documented after a 6 (3 – 9) month response. Patients who died before the 6-month response were not included in the analysis. Time was measured from the 6-month response and ended at the earliest of the date of death or last follow-up visit. A patient was considered lost to follow-up if the last visit was recorded during the first year after starting HAART and the patient had at least 1 year of additional potential follow-up until the closing date of the database. The closing date was defined for each cohort as the date of the most recent follow-up recorded in the database. Death was ascertained by local medical staff.

4.3.3 Statistical analysis

Kaplan Meier curves were used to describe time from 6-month response to HAART to death, and log-rank tests were used to test the hypothesis of equality between survival functions. Associations between independent variables and mortality were assessed by Cox proportional hazards regression with complete responders serving as the reference group. Wald tests were used to test for differences between the two types of immunologic and virologic discordant responses, using linear contrasts of the coefficients. Hazard ratios (HRs) and 95% CIs are reported. Huber-White robust standard errors were calculated to account for intra-site correlation. The proportional hazards assumption was assessed graphically by plotting scaled Schoenfeld residuals against survival time for each factor separately, and by log-log survival plots for categories of immunologic and virologic response, adjusted for other covariates. Horizontal lines at zero in the former and parallel curves in the latter test are consistent with the proportional-hazards assumption.

Preliminary analyses indicated that patients who had one measurement at 6 months (PVL or CD4 cell count) showed similar characteristics to patients who had both measurements at 6 months. Therefore missing information on 6 month PVL or CD4 cell counts were imputed for those who had only one of these measurements, based on mortality status, loss to follow-up status, site, baseline CD4 cell count, sex, age and type of HAART regime. The occurrence of an incident AIDS defining event was modeled as a time dependent covariate. The ice command in Stata was used to multiply impute missing values [103]. In these imputations, values of the missing data were randomly sampled from their predicted distributions. Analyses were run on each of 5 datasets that included the imputed values, and the results combined with Rubin's rules [95]. Analyses were performed using Stata version 9.0 (Stata Corp., College Station, TX).

4.4 RESULTS

4.4.1 Study population

A total of 20,015 antiretroviral-naïve participants from 14 sites, aged 16 years or older initiated HAART in the study period. Of these, 8,342 (41.7%) were excluded because they were missing both PVL and CD4 cell count measurements at 6 (3 - 9) months, and 293 (1.4%) were excluded because their last follow-up visit was the date of 6-month response. Compared to patients with both measurements at 6 months, individuals missing both 6-month measurements were more likely to be male, to have a lower CD4 cell count (median 99 vs. 106 cells/μL; p<.001), to be have an unknown clinical stage, and to be prescribed a non-standard HAART regimen. They also were more likely to be reported dead or lost to follow up. Compared to patients with both measurements at 6 months, individuals with one measurement were more likely to be male, to be older, to have higher baseline CD4 cell count and PVL, and to be prescribed a NNRTI-based regimen. They also were more likely to be reported to be lost to follow-up. Table 13 shows baseline characteristics and outcomes for 20,015 patients according to presence of a 6-month laboratory measurement. Kaplan-Meier survival curves are shown in Figure 2. The log-rank test for equality of survival functions showed no significant difference between the groups of patients who had one and both 6-month measures (p=.08), but a statistically significant difference between patients with both measurements vs. patients with neither measurement (p<.001).

Table 13. Patients' baseline characteristics and outcomes according to the availability of a 6-month measurement.

Variable	Total N = 20,015	Both Measured N = 5,974	1 Measured N = 5,708	Neither Measured N = 8,342	P value Both Measured vs. Neither Measured	P value Both Measured vs. 1 Measured
Baseline characteristics						
Male gender n (%)	8,835 (44.1)	2,353 (39.4)	2,520 (44.2)	3,962 (47.5)	<.001	<.001
Age years (IQR)	35 (30 – 41)	34 (30 – 41)	35 (30 – 41)	35 (30 – 40)	<97	<.001
Baseline CD4 count cells/μL (IQR)	106 (46 – 180)	106 (46 – 179)	116 (53 – 189)	99 (40 – 173)	<.001	<.001
Baseline PVL log_{10} copies/mL (IQR)	4.99 (4.41 – 5.46)	4.98 (4.43 – 5.47)	5.04 (4.43 – 5.53)	4.99 (4.31 – 5.44)	.07	.23
Clinical stage n(%)					<.001	<.001
Less advanced	1,699 (8.5)	722 (12.1)	563 (9.9)	414 (5.0)		
Advanced	16,545 (82.7)	4,853 (81.3)	4,630 (81.2)	7,062 (84.6)		
Unknown	1,771 (8.8)	394 (6.6)	511 (8.9)	866 (10.4)		
Regimen type n (%)					<.001	<.001
NNRTI based	16,517 (82.5)	4,809 (80.6)	4,850 (85.0)	6,858 (82.2)		
PI based	2,069 (10.3)	864 (14.5)	429 (7.5)	776 (9.3)		
Other	1,429 (7.2)	296 (4.9)	425 (7.5)	708 (8.5)		
Outcomes						
Mortality rate per 100PY (95% CI)	3.92 (3.72 – 4.14)	1.42 (1.22 – 1.64)	1.75 (1.53 – 2.00)	10.43 (9.78 – 11.12)	<.001	.08
Loss to follow-up n (%)	3,095 (15.5)	569 (9.5)	662 (11.6)	1,864 (22.4)	<.001	<.001

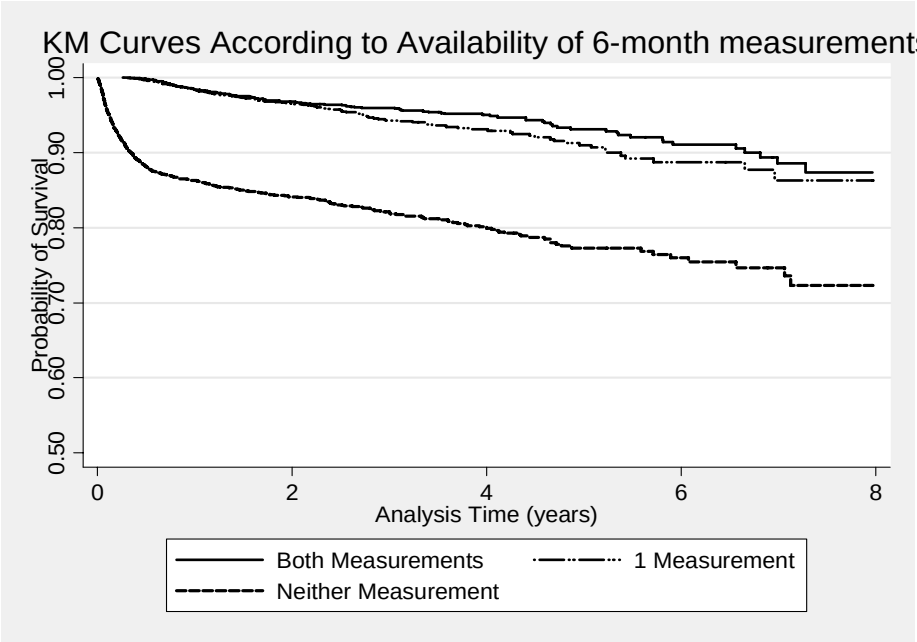


Figure 2. Kaplan-Meier survival curves according to availability of 6-month measurements

For the subsequent analysis, we excluded patients with neither measurement at 6 months. Figure 3 shows the disposition of patients included in the all-cause mortality analysis.

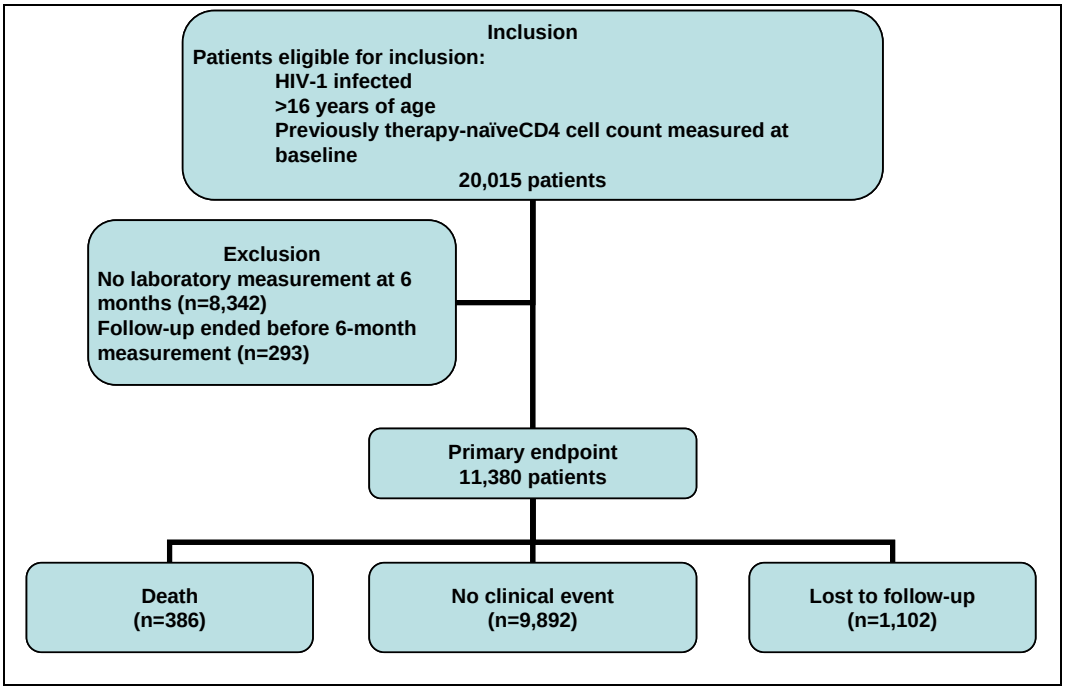


Figure 3. Patient disposition and outcomes

Of the 11,380 patients included in the present analysis, 4,739 (41.6%) were male, and the median age was 35 [interquartile range (IQR) 30 – 41)]. The median baseline CD4 cell count was 110 (IQR 49 – 184) cells/μL, and median PVL was 4.90 (IQR 4.25 – 5.50) $\log_{10}$ copies/mL. The majority of the patients (81.5%) was at advanced stage of disease and was prescribed a NNRTI-based regimen as first line therapy (82.7%). At 6 (3 – 9) months, 7,181 patients (63.1%) showed complete response; 1,710 (15.0%) had virologic-only response; 1,653 (14.5%) had immunologic-only response; and 836 (7.4%) showed no response. Baseline characteristics of the patients according to immunologic and virologic responses at 6 months are shown in Table 14. At baseline, virologic-only responders had the highest CD4 cell count and the lowest PVL, whereas immunologic-only responders had the lowest CD4 cell count and the highest PVL. Virologic-only responders tended to be older than patients from other categories of response.

Table 14. Patients' baseline characteristics and outcomes according to immunologic and virologic responses at 6 months of therapy.

Variable	Total N = 11,380	VR+IR+ N = 7,181	VR+IR- N = 1,710	VR-IR+ N = 1,653	VR-IR- N = 836
Baseline characteristics					
Male gender n (%)	4,739 (41.7)	2,804 (39.0)	747 (43.7)	750 (45.4)	438 (52.4)
Age years (IQR)	35 (30 – 41)	35 (30 – 41)	36 (31 – 43)	34 (29 – 40)	35 (30 – 41)
Baseline CD4 count cells/μL (IQR)	110 (49 – 184)	103 (47 – 175)	147 (75 – 238)	95 (41 – 170)	131 (63 – 214)
Baseline PVL $\log_{10}$ copies/mL (IQR)	4.90 (4.25 – 5.50)	4.94 (4.30 – 5.53)	4.60 (3.91 – 5.21)	5.06 (4.39 – 5.70)	4.85 (4.14 – 5.49)
Clinical stage n(%)					
Less advanced	1,254 (11.0)	822 (11.4)	231 (13.5)	138 (8.4)	63 (7.5)
Advanced	9,277 (81.5)	5,846 (81.4)	1,343 (78.5)	1,389 (84.0)	702 (84.0)
Unknown	849 (7.5)	516 (7.2)	136 (8.0)	126 (7.6)	71 (8.5)
Regimen type n (%)					

Table 14 continued:

Variable	Total N = 11,380	VR+IR+ N = 7,181	VR+IR- N = 1,710	VR-IR+ N = 1,653	VR-IR- N = 836
NNRTI based	9,410 (82.7)	6,266 (87.3)	1,453 (85.0)	1,164 (70.4)	527 (63.0)
PI based	1,262 (11.1)	640 (8.9)	168 (9.8)	274 (16.6)	180 (21.5)
Other	708 (6.2)	275 (3.8)	89 (5.2)	215 (13.0)	129 (15.4)
Outcomes					
Mortality rate per 100PY (95% CI)	2.00 (1.81 – 2.21)	1.31 (1.12 – 1.54)	3.03 (2.43 – 3.78)	2.02 (1.60 – 2.54)	4.98 (4.03 – 6.16)
Loss to follow-up n (%)	1,102 (9.7)	548 (7.6)	140 (8.2)	247 (14.9)	167 (20.0)

4.4.2 All-cause mortality

A total of 386 deaths were reported during 19,295 person-years (PY), corresponding to a mortality rate of 2.00 (95% CI 1.81 – 2.21) per 100 PY (Table 14). The mortality rate was highest among non-responders, followed by virologic-only, immunologic-only, and complete responders. The overall rate of loss-to-follow-up was 9.7%, and was highest among non-responders (20.0%). Table 15 summarizes mortality rates and median follow-up times for each of the 14 participating sites, and the corresponding geographic regions. The mortality rates varied across sites, and were generally higher in Africa. The median follow-up time was 1.15 years (IQR 0.60 – 2.12), and varied across sites. The two Brazilian sites had the longest follow-up times (median 4.03 [IQR 1.14 – 6.19]; and 4.25 [IQR 1.83 – 6.44] years for Rio de Janeiro and Porto Alegre, respectively). Figures 4, 5 and 6 show the Kaplan-Meier survival curves of participating cohorts by region.

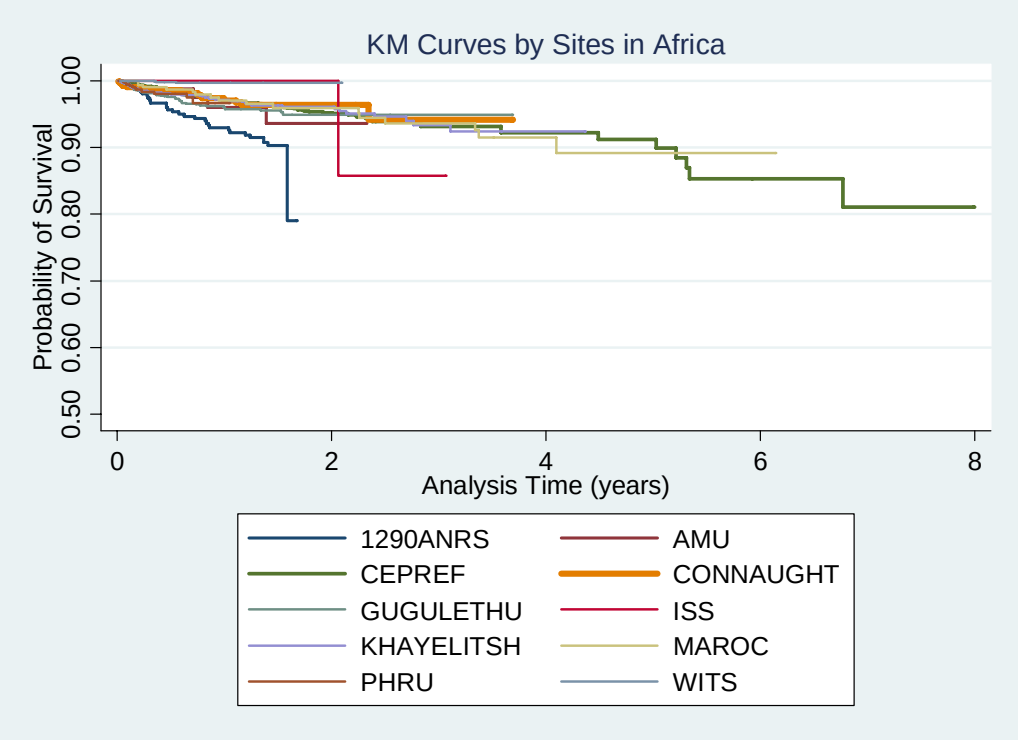


Figure 4. Kaplan-Meier estimates of probability of survival of cohorts in Africa

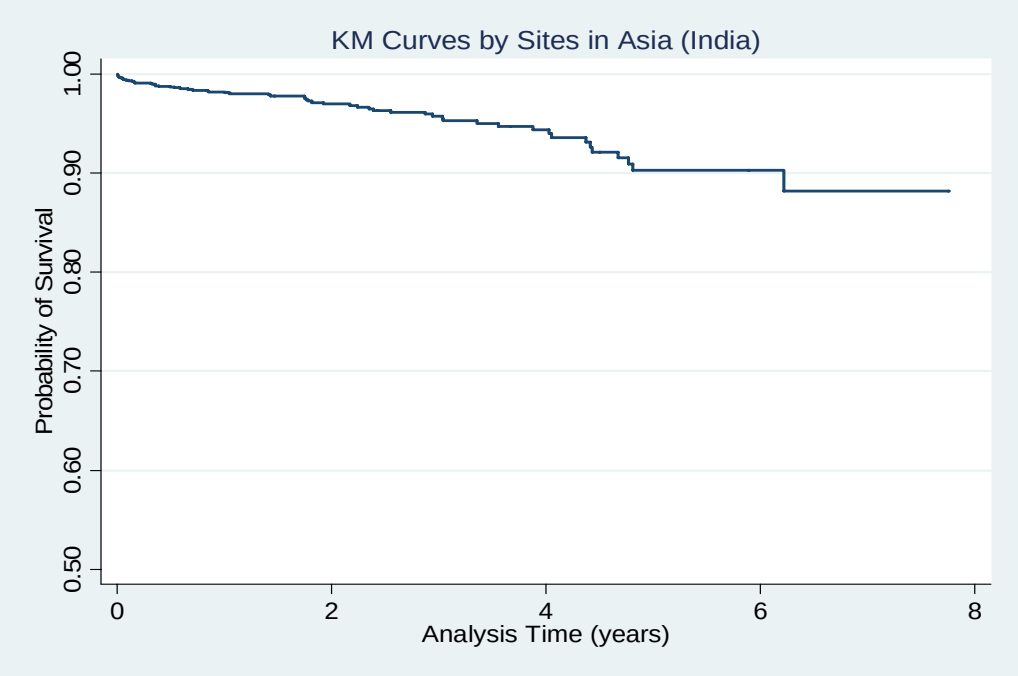


Figure 5. Kaplan-Meier estimates of probability of survival of the cohort in Asia (India)

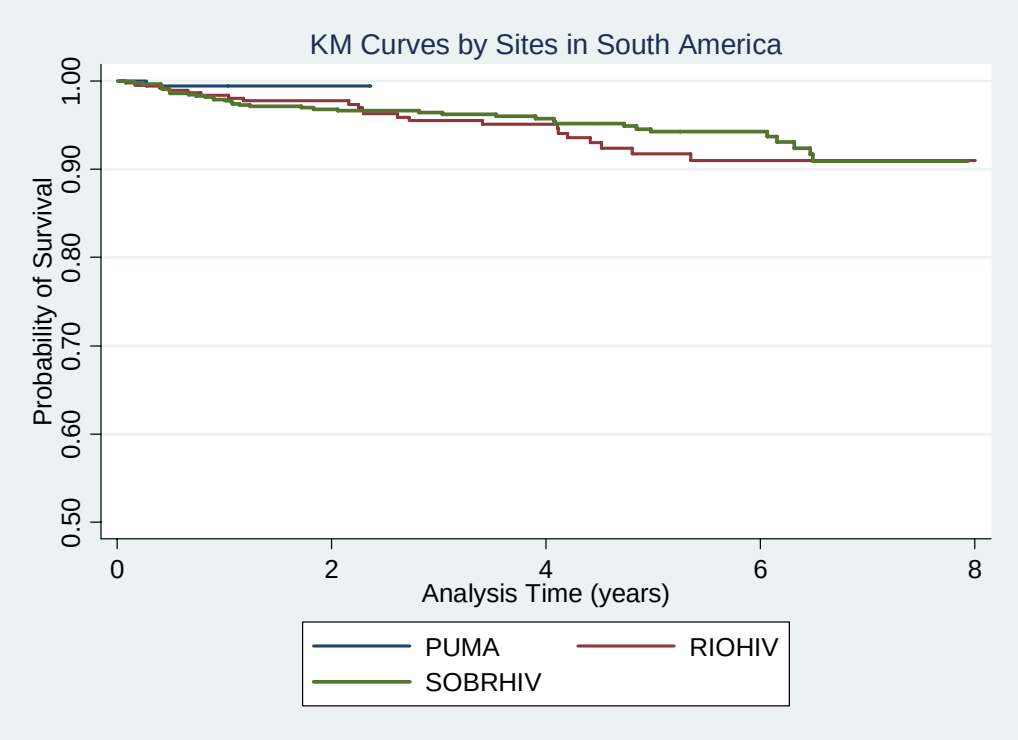


Figure 6. Kaplan-Meier estimates of probability of survival of cohorts in South America

Table 15. Site-specific mortality and follow-up for the 14 participating ART-LINC sites

Cohort	Mortality rate per 100 PY (95%CI)	Median follow up time in years (IQR)	Loss to follow up rate (%)
1290ANRS (Côte d'Ivoire)	7.14 (4.96 – 10.28)	1.48 (1.41 – 1.50)	55.8
AMU (Uganda)	3.55 (1.33 – 9.46)	1.39 (0.99 – 1.90)	2.5
CEPREF (Côte d'Ivoire)	2.55 (2.07 – 3.14)	1.68 (0.92 – 2.26)	2.7
CONNAUGHT (Zimbabwe)	2.49 (1.57 – 3.96)	0.99 (0.57 – 1.68)	2.0
GUGULETHU (South Africa)	3.26 (2.43 – 4.38)	0.92 (0.34 – 1.53)	2.0
ISS (Uganda)	3.13 (0.44 – 22.21)	0.33 (0.15 – 1.30)	17.5
KHAYELITSH (South Africa)	2.64 (2.00 – 3.49)	1.02 (0.55 – 1.67)	3.1
MAROC (Morocco)	2.41 (1.47 – 3.93)	1.36 (0.79 – 3.41)	48.7
PHRU (South Africa)	4.21 (2.39 – 7.42)	0.52 (0.38 – 0.69)	0.2
PUMA (Argentina)	0.55 (0.07 – 3.91)	0.90 (0.52 – 1.28)	14.4
RIOHIV (Brazil)	1.30 (0.86 – 1.98)	4.03 (1.14 – 6.19)	6.7
SOBRHIV (Brazil)	1.02 (0.74 – 1.39)	4.25 (1.83 – 6.44)	18.2

Table 15 continued:

Cohort	Mortality rate per 100 PY (95%CI)	Median follow up time in years (IQR)	Loss to follow up rate (%)
WITS (South Africa)	0.30 (0.11 – 0.82)	0.94 (0.63 – 1.14)	2.1
YRGCARE (India)	1.70 (1.31 – 2.20)	1.43 (0.50 – 3.03)	21.7
Region	**Mortality rate per 100 PY (95%CI)**	**Median follow up time in years (IQR)**	**Loss to follow up rate (%)**
Africa	2.61 (2.31 – 2.94)	1.04 (0.57 – 1.64)	6.2
Asia (India)	1.70 (1.32 – 2.20)	1.43 (0.50 – 3.03)	21.7
South America	1.09 (0.85 – 1.39)	3.50 (1.15 – 6.08)	14.4

The Kaplan-Meier survival curves of categories of immunologic and virologic responses (complete, virologic only, immunologic only, and absent) are shown in Figure 4. The log-rank test for equality of survival functions showed significant differences between these groups ($p<.001$), with the complete responders having the best survival and the non-responders having the worst survival. Figures 5, 6 and 7 show the corresponding Kaplan-Meier survival curves for the 3 regions (Africa, Asia, and South America). These figures show the same general pattern for complete responders and non-responders across regions, with some inconsistent relationships between the two categories of discordant responses. Life tables with probability of remaining alive by a given time and corresponding 95% CI for each category combination of immunologic and virologic responses are shown in the Appendix.

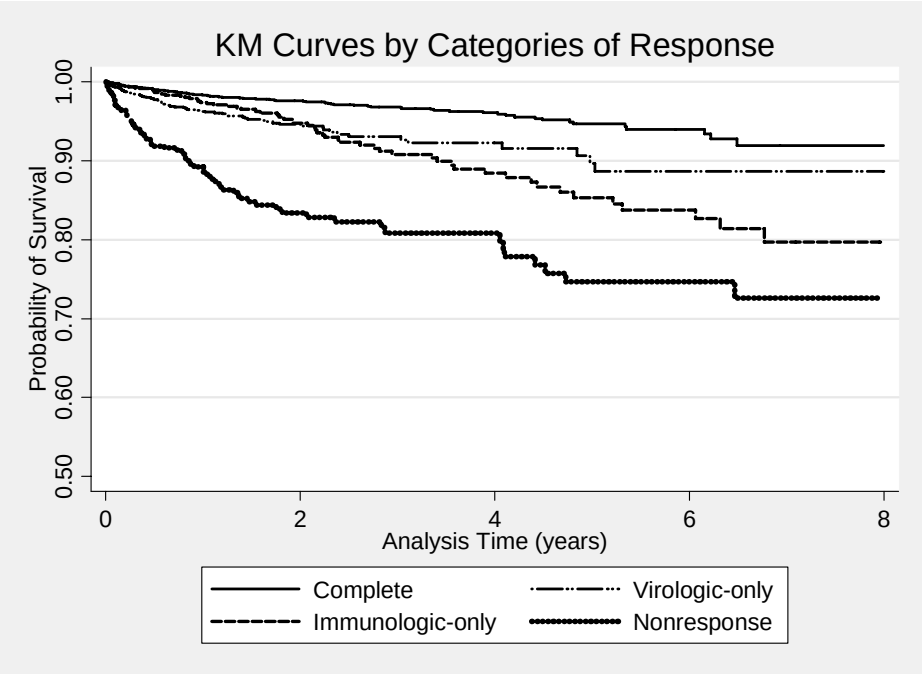


Figure 7. Kaplan-Meier estimates of probability of survival according to categories of immunologic and virologic responses at 6 months in 11, 380 patients.

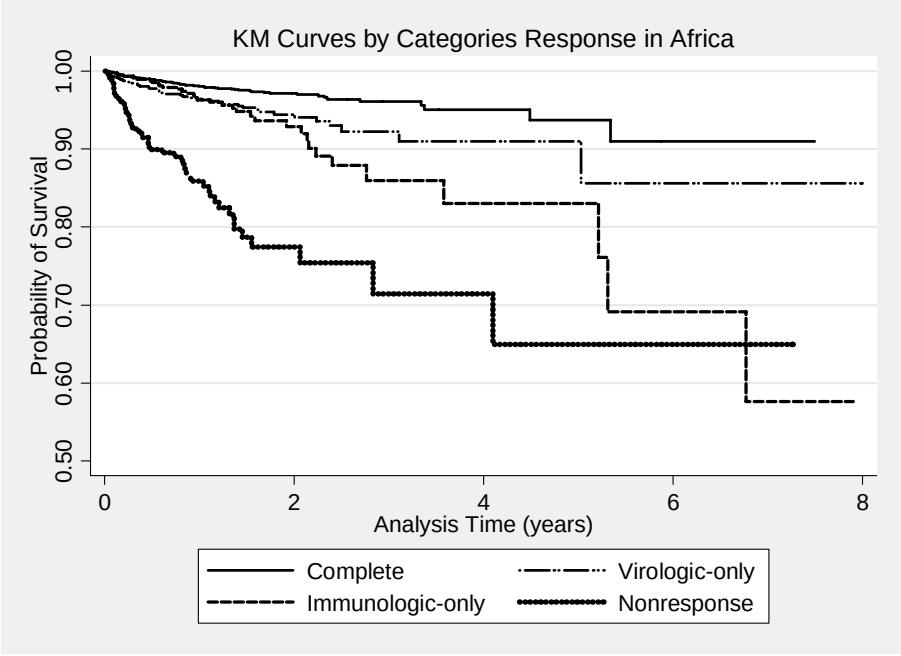


Figure 8. Kaplan-Meier estimates of probability of survival according to categories of immunologic and virologic responses at 6 months in Africa.

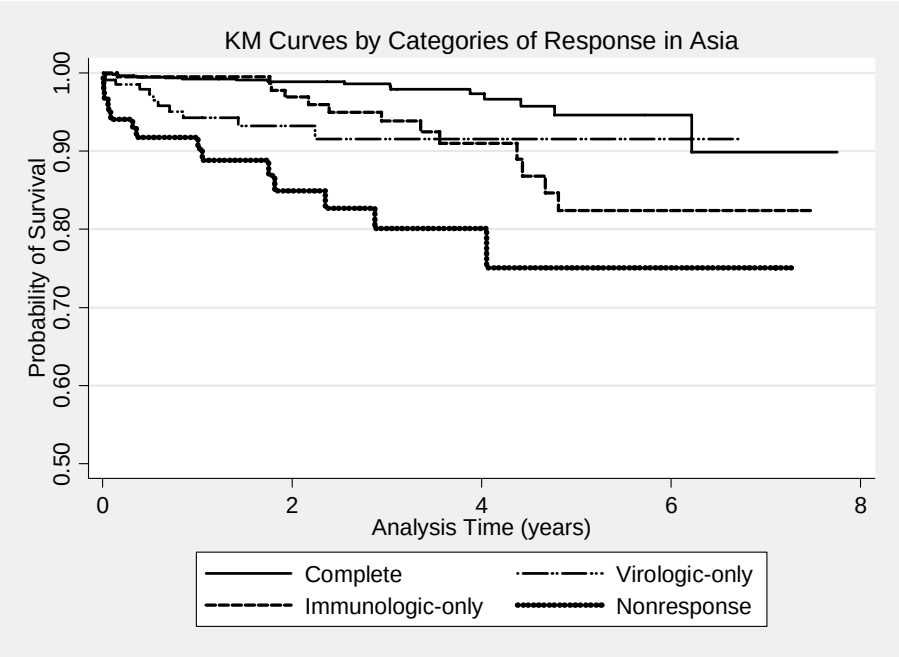


Figure 9. Kaplan-Meier estimates of probability of survival according to categories of immunologic and virologic responses at 6 months in Asia.

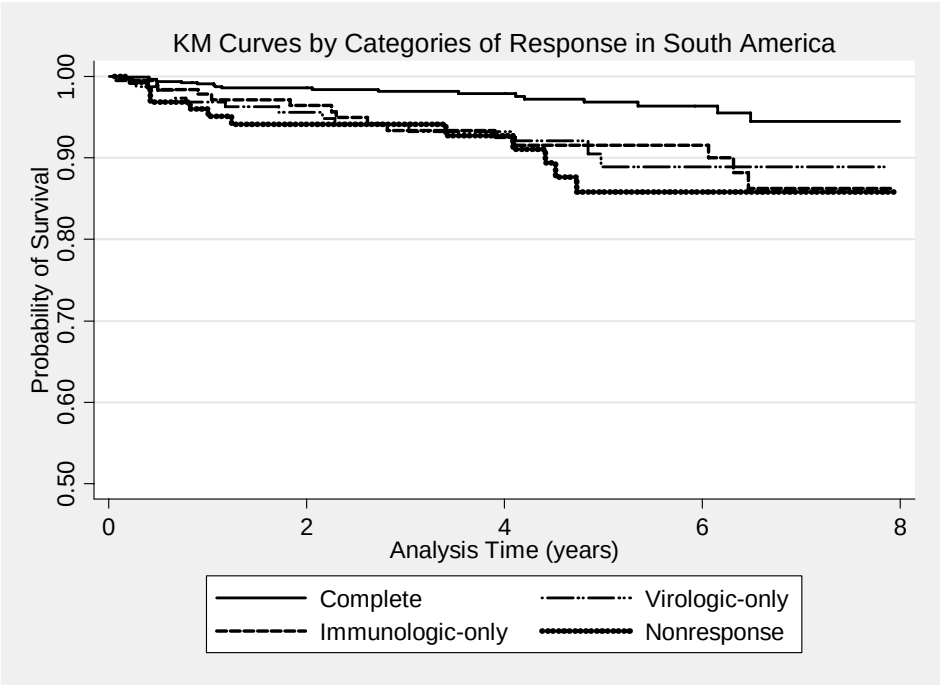


Figure 10. Kaplan-Meier estimates of probability of survival according to categories of immunologic and virologic responses at 6 months in South America.

In multivariable analysis adjusted for age at HAART initiation, clinical stage, baseline CD4 cell count, history of an AIDS defining condition after the 6 month response, cohort, and year of HAART initiation, both categories of discordant immunologic and virologic responses and non-responses were associated with increased risk of death. Compared with complete responders, virologic-only responders had a HR of 2.29 (95% CI 1.64 – 3.20), immunologic-only responders had a HR of 2.22 (95% CI 1.52 – 3.26), and non-responders had a HR of 6.21 (95% CI 4.23 – 9.13). Age greater than 50 years was also associated with increased risk of death (HR compared to 16 – 29 year category: 1.74; 95% CI 1.27 – 2.38). A higher baseline CD4 cell count was independently associated with improved survival (HR for increase in 100 cells/μL 0.73; 95% CI 0.65 – 0.83), even after adjusting for the 6 month response. Having an AIDS defining condition was associated with a hazard of 6.43 (95% CI 4.40 – 9.39). The two types of discordant immunologic and virologic responses were significantly different (Wald test p = .016). Plots of scaled Schoenfeld residuals for each variable included in the final model did not show violation of the proportional hazards assumption (Appendix). Results from univariate and multivariable analyses are summarized in Table 16.

Table 16. Unadjusted and adjusted Cox proportional hazards and 95% confidence intervals of all-cause mortality for 11,380 patients*

Variable	Unadjusted HR	P	Adjusted HR	P
6-month response				
Complete (ref)	1.00		1.00	
Virologic-only	2.30 (1.65 – 3.19)	<.001	2.29 (1.64 – 3.20)	<.001
Immunologic-only	1.71 (1.28 – 2.28)	<.001	2.22 (1.52 – 3.26)	<.001
Absent	4.26 (2.94 – 6.16)	<.001	6.21 (4.23 – 9.13)	<.001

Table 16 continued:

Variable	Unadjusted HR	P	Adjusted HR	P
Baseline CD4 cell count (100cells increase/μL)	0.78 (0.68 – 0.89)	<.001	0.73 (0.65 – 0.83)	<.001
Age				
16 – 29 (ref)	1.00		1.00	
30 – 39	1.08 (0.88 – 1.33)	.429	1.07 (0.85 – 1.35)	.539
40 – 49	1.19 (0.95 – 1.48)	.124	1.12 (0.93 – 1.36)	.233
50+	1.64 (1.28 – 2.09)	<.001	1.74 (1.27 – 2.38)	.001
Clinical stage				
Less advanced (ref)	1.00		–	
Advanced	1.21 (0.80 – 1.83)	.352	–	
Unknown	1.26 (0.81 – 1.98)	.298	–	
AIDS defining event	6.73 (4.96 – 9.13)	<.001	6.43 (4.40 – 9.39)	<.001
ARV Regimen				
NNRTI based (ref)	1.00		–	
PI-based	1.13 (0.78 – 1.63)	.509	–	
Other	1.09 (0.65 – 1.84)	.742	–	

*Adjusted for year of therapy initiation and site as fixed effects

4.5 DISCUSSION

We report on the impact of immunologic and virologic discordant responses at 6 months after HAART initiation in a large collaboration of cohorts from lower income countries. Our analysis showed that both types of discordant responses are associated with increased risk of death

relative to complete responders, which is in agreement with previous reports from developed countries [20, 22, 25].

Understanding the relationship between early responses to HAART and mortality has critical implications for guiding treatment modifications, particularly for those patients who show discordant immunologic and virologic responses. In resource-limited settings, where second- and third-line regimen options as well as access to laboratory tests often are limited, the additional problem of background morbidity may pose a challenge for developing treatment guidelines. We previously reported that incidence of discordant immunologic and virologic responses in resource-constrained countries were similar to that observed in resource-rich settings [104]. In this analysis, we assessed whether risk of death was increased in patients showing discordant immunologic and virologic responses at 6 months of HAART in the same population.

Our results are similar to those reported by Moore et al.[22], who assessed the independent association of discordant responses with mortality in 2,217 ARV-naïve individuals initiating HAART in British Columbia. These authors also found that discordant responses were associated with increased risk of death when compared with complete responders. Our results are also in line with those of Piketty et al., who reported that immunologic-only responders had a better outcome than virologic-only responders in a study involving 150 HIV-infected individuals [25].

One concern that arises from the wide use of NNRTI-based regimens is that qualitative differences in immunologic-only response could exist between PI and NNRTI recipients due to different genetic barriers to resistance. One could hypothesize that lack of virologic suppression in the former group would be due to reduced viral fitness [45, 89], while in the latter group

would be due to genotypic resistance, which, in turn, could be associated with a worse outcome. A study of 1,138 previously antiretroviral therapy naive HIV-infected individuals from British Columbia showed that patients who developed resistance to NNRTI had a risk 3.02 times higher than those who had no resistance [105]. Although we did not find an association between regimen type and mortality in our analysis, direct comparisons of different regimens in observational studies are subject to selection bias. Further studies are needed to clarify the association of different patterns of immunologic-only response between recipients of PI vs non-PI regimens, particularly with respect to the emergence of resistance.

Of note, our analysis did not confirm the superiority of 6-month response over baseline values for predicting clinical progression or death, as studies from developed countries have previously demonstrated. In a large collaboration of cohorts from Europe and North America, baseline CD4 and HIV-1 RNA were not associated with disease progression or death after controlling for 6-month values [69], suggesting that even advanced immune suppression could be overcome with HAART. Similar findings are reported by Anastos et al. among HIV-infected women in the USA [18]. In ART-LINC, although 6-month values were predictive of death or clinical progression, baseline CD4 count remained a strong predictor in the final model. Indeed, it has been increasingly argued that the degree of initial immunologic damage has profound long-term consequences. A study of patients who maintained sustained virologic suppression for up to 6 years showed that although the initial CD4 cell gain is higher among those with lower baseline values, only patients with a baseline value of > 350 cells/μL had CD4 cell counts returned to nearly normal values at 6 years [106]. Studies from South [107] and West Africa [108] support the notion that, in these regions, HAART should be considered before CD4 cell count drops to levels <350 cells/μL, due to the high morbidity and mortality observed among these patients. It

appears that severe immunodepression, perhaps associated with other clinical conditions, is not overcome by HAART in resource-limited as it is in resource-rich settings, and that specific prognostic models need to be developed for resource-limited settings.

Our study has several limitations. First, data on adherence were not available in our sample. Several studies have shown the independent association between poor adherence and increased mortality [22, 109, 110]. Second, other possible confounders could not be assessed in our study. For example, studies on the pathogenesis of immunologic-only response have suggested possible roles of factors associated with viral subtypes, such as non-syncytion-inducing (CCR5-tropic) viruses [46], reduced viral fitness [45], decreased T-cell activation [111, 112], and host-related determinants such as genetic polymorphisms [39]. Poor CD4 cell recovery has been associated with regimens containing didanosine plus tenofovir [99], and polymorphisms of interleukin-6 and central major histocompatibility complex genes [38]. Third, the exclusion of nearly 40% of patients due to lack of information on the 6-month response potentially could have introduced a bias into our results. Excluded patients due to missing of 6-month response differed with respect to some baseline characteristics, were more likely to be lost to follow-up, and showed worse outcomes than those remaining in the analysis. Arguably, having at least one laboratory measure taken at 6 months is a marker of better compliance to service and, consequently, to treatment. Because we could not make any inferences about missing patterns of those who missed both types of response, we decided to exclude them from the analysis. Finally, we also noticed a tendency of higher loss to follow-up rate among non-responders. These patients also were more likely to die, so this finding could be indicative of a selection bias and could have led us to underestimate the hazard for this group. Therefore, our results can be generalized to patients from lower income countries who have a 6-month laboratory assessment.

In conclusion, we found in ART-LINC that both types of immunologic and virologic discordant responses were predictors of mortality when compared with complete responses. This is the largest study to date using pooled data from resource-limited countries, where most of naïve patients are initiating HAART with NNRTI-based regimens. Most studies published so far have been conducted in developed countries and have included patients using PI-based regimens. Our results suggest that, similar to resource-rich settings, both immunologic and virologic assessment are important for predicting mortality in patients receiving HAART in resource-constrained countries, and provide a strong argument for recommending the wider availability of plasma viral load testing to guide therapy switch in these settings.

5.0 GENERAL DISCUSSION AND PUBLIC HEALTH IMPORTANCE

The global goal of reversing the picture of HIV epidemic by 2015, through radically scaling up prevention, treatment and care has required a substantial amount of funding to expand programmes and increase antiretroviral therapy coverage rates. Yet, nearly 5 million people still are in need of therapy, and the sustainability of scaling-up strategies remains questionable, as there is scarce evidence base to guide policy in low- and middle-income countries. Although available data on treatment are improving gradually, more extensive knowledge about treatment impact, toxicities, and drug resistance will be needed to guide the scale-up and allow sustainability of treatment programmes.

Several factors that are particular to the countries where AIDS has struck most heavily, such as high prevalence of background infectious diseases, structural and cultural barriers, and even population and virus genetic patterns potentially could affect the effectiveness of HAART. Thus, prognostic models and treatment guidelines that are based on data from resource-rich settings likely are not suitable for guiding public health strategies in resource-limited settings. Initiatives such as ART-LINC and IeDEA are of capital importance to provide answers and insights on this issue. Here we presented four papers that assess the effectiveness of HAART using data from ART-LINC.

The first paper is a literature review of immunologic and virologic discordant reponses to antiretroviral therapy. Although there is a growing interest on the issue, the lack of a universal definition for discordance makes it difficult to establish meaningful comparisons between studies. The second paper assessed the effectiveness of HAART – measured as a virologic suppression after 6 months on therapy, in a Brazilian cohort – and found it to be similar to developed countries. We also showed that response rates improved over time, which could be attributed to the introduction of more potent antiretroviral drugs that also allowed a better

adherence. The Brazilian experience has been seen as a model for understanding many aspects of the HIV epidemic in resource-constrained settings and provided important lessons for other developing countries that aim to provide universal access to HAART. However, Brazil now faces the challenge of sustaining the policy of universal access to antiretrovirals as the number of patients on treatment is increasing and so is the need for more complex and costly regimens [113, 114]. Nevertheless, Brazil's AIDS treatment model resulted in sustained lower prices, saving Brazil over US$1 billion from 2001 to 2005 [115].

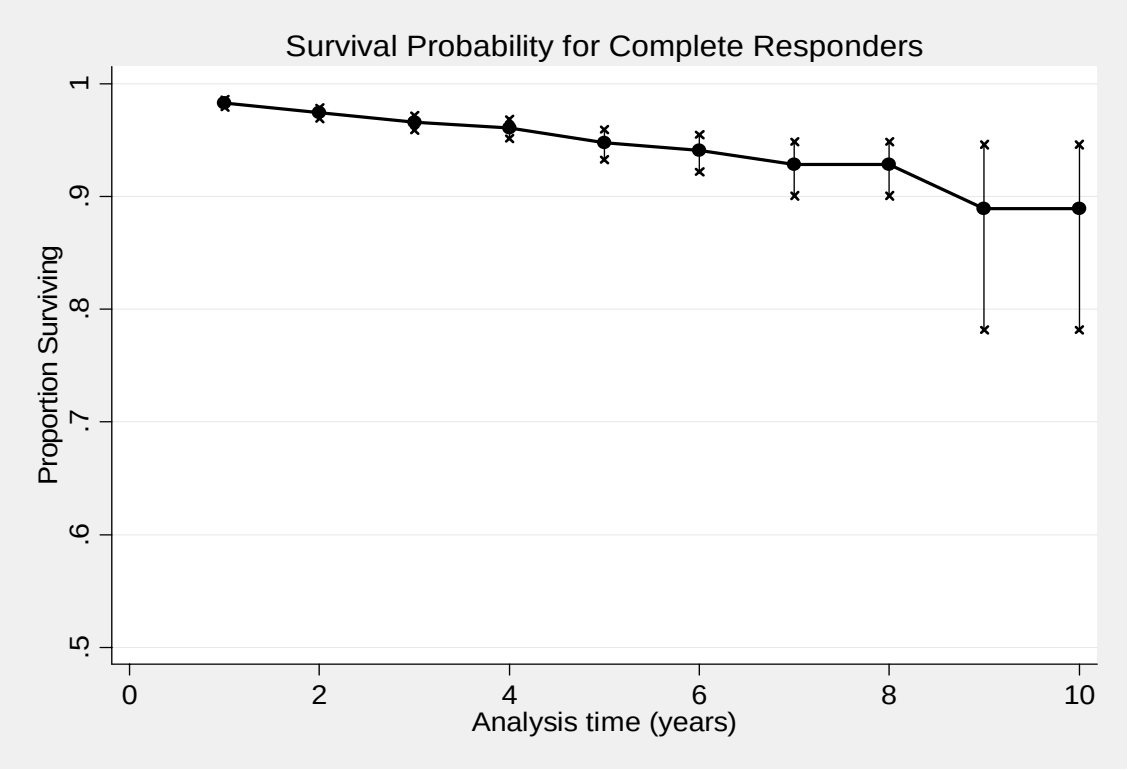
In the third and fourth papers, we sought to assess the additional prognostic value of immunologic response in predicting clinical progression in ART-LINC. We first assessed the prevalence of and risk factors for immunologic and virologic responses (categorized as complete, virologic-only, immunologic-only, and absent), and found that they were similar to those reported in studies from resource-rich countries. We have found that, compared to complete responders, virologic only responders were older, had higher baseline CD4 counts, lower baseline viral load, and were more likely to have received a nonstandard HAART regimen; and immunologic only responders were younger, had a lower baseline CD4 count, and were more likely to have received a PI based or a nonstandard regimen. Higher baseline CD4 count was associated with an increased probability of a virologic only response and with a reduced probability of an immunologic only response [20, 88, 98]. One possible explanation for this finding is the non-linear nature of CD4 cell increase after HAART initiation across the different baseline CD4 count strata. In addition, the definition of immunologic response in this and other studies (increase in CD4 cell count by 50 cells) may be subject to a regression towards the mean effect, by which those at the highest baseline values would be less likely to show improvement than those with low baseline values [116]. The fourth paper showed that both types of

discordant responses predict of clinical progression and mortality. However, we noticed a high proportion of patients who did not have a 6-month PVL and CD4 cell count measurement. This group of patients also had higher mortality and loss-to-follow-up rate, which led us to hypothesize that not having at least one measurement at 6 months of therapy was an indirect marker for less access to care and for other factors associated with worse outcome. The crude mortality rates for each of the four categories of immunologic and virologic response were also lower than the reported in the literature and are suggestive of a survival bias. This hypothesis is supported by another analysis done in this population, where cohorts with active tracing of patients showed a higher mortality than cohorts that had a passive follow-up system [58], suggesting that death is under ascertained in many cohorts participating in ART-LINC. In addition, ART-LINC sites may overrepresent 'centres of excellence', as eligibility criteria for participating in the collaboration include having electronic data collection capabilities. Lack of a minimal infrastructure to reliably record information on outcomes as well as high rates of loss to follow up are major obstacles to the conduct of high-impact research in these settings.

In summary, we have demonstrated that effectiveness of HAART in low- and middle-income countries is similar to that observed in resource-rich countries, provided that access to care is comparable. We also found differences between resource-limited and -rich countries and across ART-LINC sites that warrant additional research. Public health decisions about treatment guidelines must be driven by sound evidence of health effects, but still have to be weighted by aspects such as feasibility and contextual factors. Statistical models accounting for the hierarchical structure of individual and contextual factors are needed to better understand the potential impact of these factors, and will help develop prognostic models and treatment guidelines applicable to this setting.

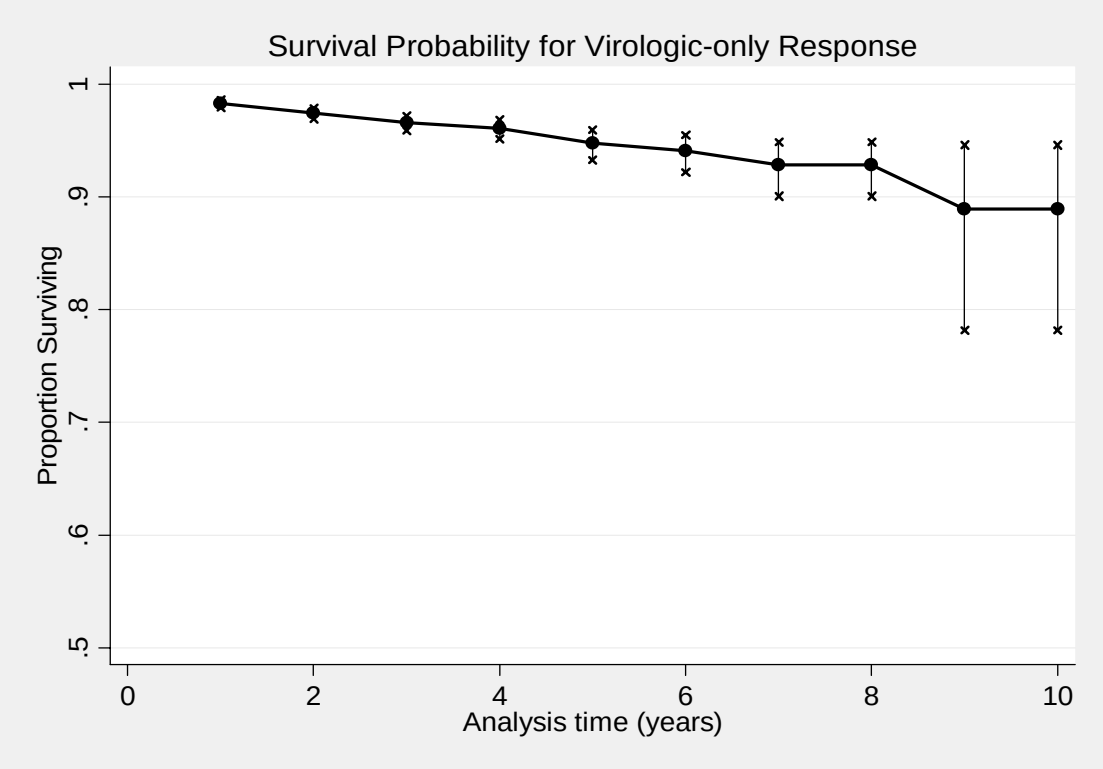
APPENDICES

APPENDIX A. Life-table, and survival probability plots with 95% confidence intervals for complete responders



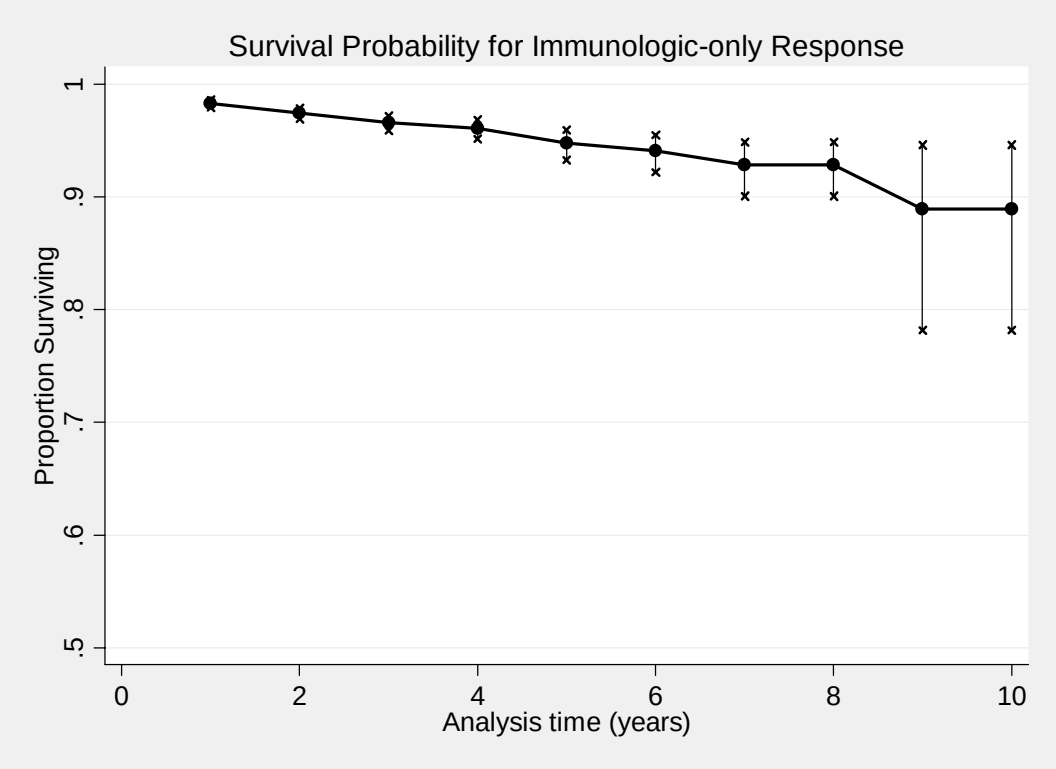
Interval (years)	Total at the beginning	Events	Persons at risk	Probability of death	95%CI	
0 – 1	7181	97	3024	0.9829	0.9792	0.986
1 – 2	4060	26	2200	0.9743	0.9691	0.9786
2 – 3	1834	12	908	0.9658	0.9584	0.9719
3 – 4	914	4	363	0.9605	0.9512	0.9681
4 – 5	547	6	203	0.9476	0.9326	0.9593
5 – 6	338	2	133	0.9406	0.922	0.9549
6 – 7	203	2	105	0.9281	0.9005	0.9483
7 – 8	96	0	55	0.9281	0.9005	0.9483
8 – 9	41	1	34	0.8894	0.7814	0.9458
9 – 10	6	0	6	0.8894	0.7814	0.9458

APPENDIX B. Life-table, and survival probability plots with 95% confidence intervals for virologic-only responders



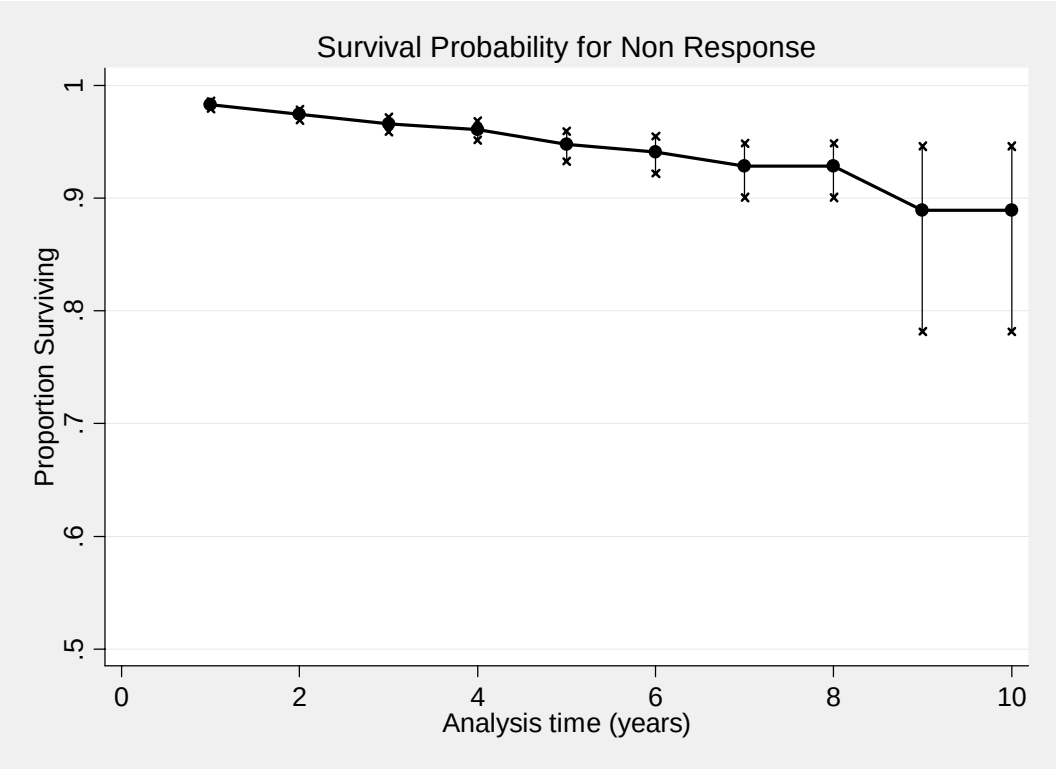
Interval (years)	Total at the beginning	Events	Persons at risk	Probability of death	95%CI	
0 – 1	1710	57	777	0.95	0.94	0.96
1 – 2	876	10	481	0.94	0.92	0.95
2 – 3	385	6	165	0.92	0.90	0.94
3 – 4	214	2	87	0.91	0.88	0.93
4 – 5	125	3	38	0.88	0.84	0.91
5 – 6	84	0	27	0.88	0.84	0.91
6 – 7	57	0	27	0.88	0.84	0.91
7 – 8	30	0	17	0.88	0.84	0.91
8 – 9	13	0	9	0.88	0.84	0.91
9 – 10	4	0	4	0.88	0.84	0.91

APPENDIX C. Life-table and survival probability plots with 95% confidence intervals for immunologic-only responders



Interval (years)	Total at the beginning	Events	Persons at risk	Probability of death	95%CI	
0 – 1	1653	29	539	0.97	0.97	0.98
1 – 2	1085	13	464	0.96	0.95	0.97
2 – 3	608	12	229	0.94	0.92	0.95
3 – 4	367	6	74	0.92	0.89	0.94
4 – 5	287	6	67	0.90	0.87	0.92
5 – 6	214	2	58	0.89	0.85	0.91
6 – 7	154	5	56	0.85	0.80	0.89
7 – 8	93	0	47	0.85	0.80	0.89
8 – 9	46	0	38	0.85	0.80	0.89
9 – 10	8	0	8	0.85	0.80	0.89

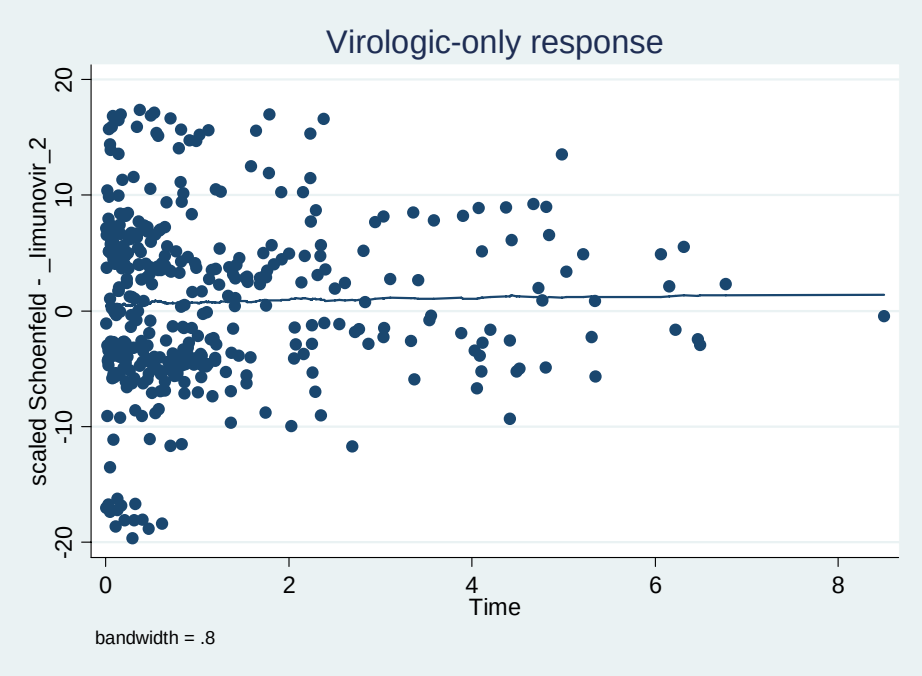
APPENDIX D. Life-table and survival probability plots with 95% confidence intervals for nonresponders

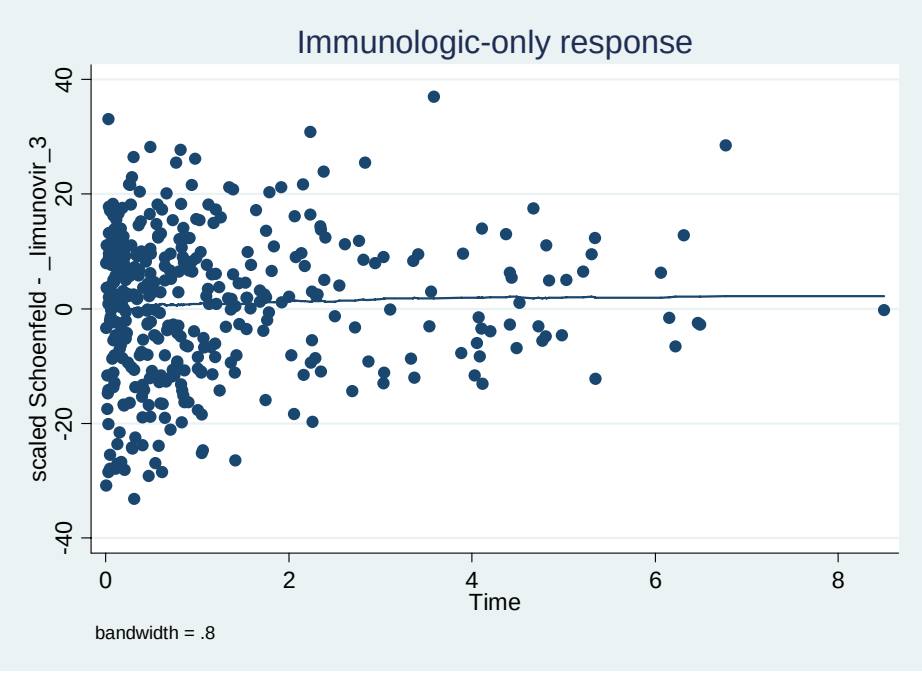


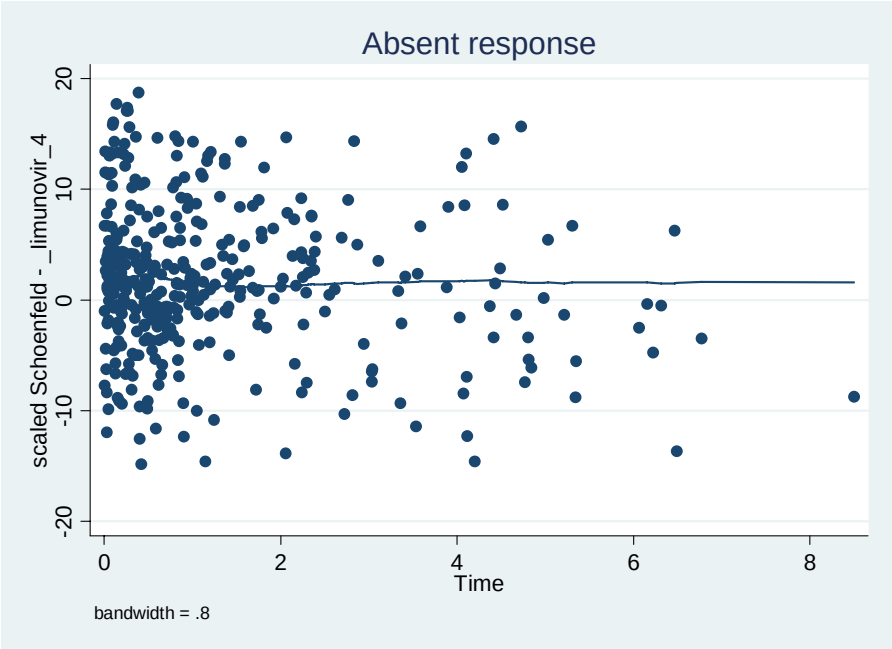
Interval (years)	Total at the beginning	Events	Persons at risk	Probability of death	95%CI	
0 – 1	836	57	311	0.92	0.89	0.93
1 – 2	468	16	188	0.88	0.85	0.90
2 – 3	264	4	90	0.86	0.83	0.89
3 – 4	170	1	30	0.86	0.82	0.89
4 – 5	139	6	23	0.82	0.77	0.86
5 – 6	110	1	25	0.81	0.75	0.85
6 – 7	84	0	25	0.81	0.75	0.85
7 – 8	59	0	27	0.81	0.75	0.85
8 – 9	32	0	25	0.81	0.75	0.85
9 – 10	7	0	7	0.81	0.75	0.85

APPENDIX E. Plots of scaled Schoenfeld residuals for variables included in the final model

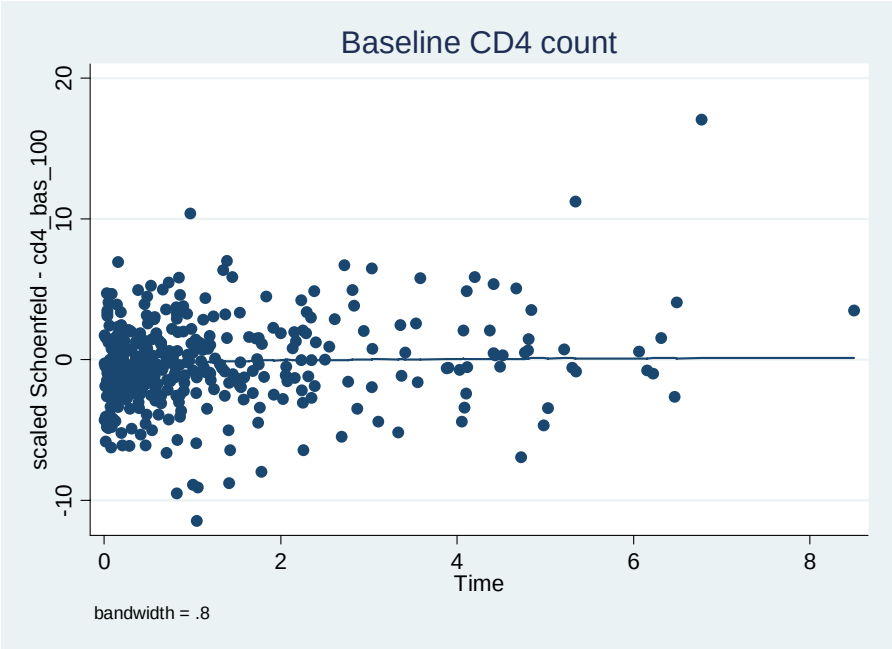
a) Categories of immunologic and virologic responses



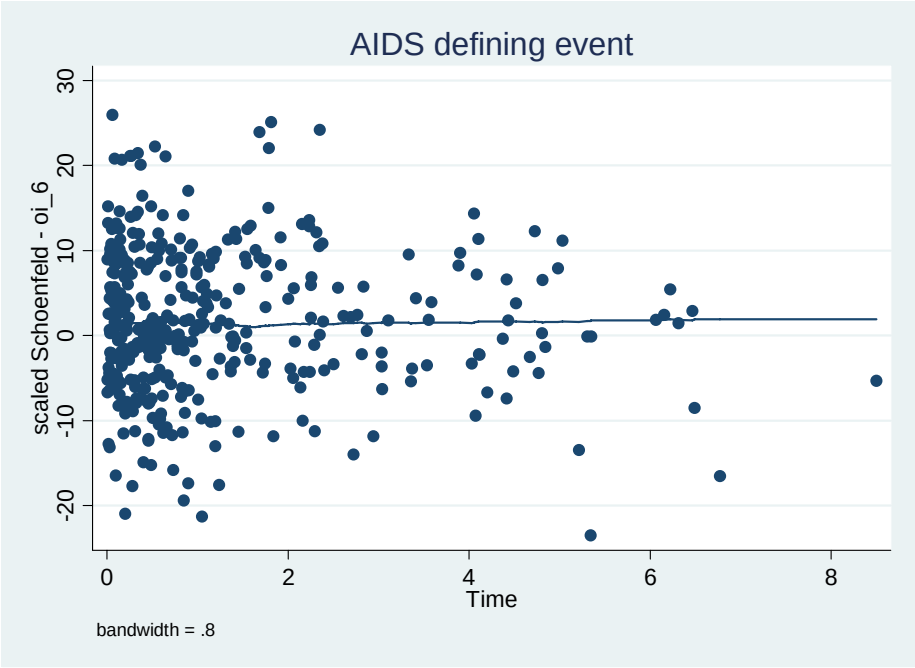




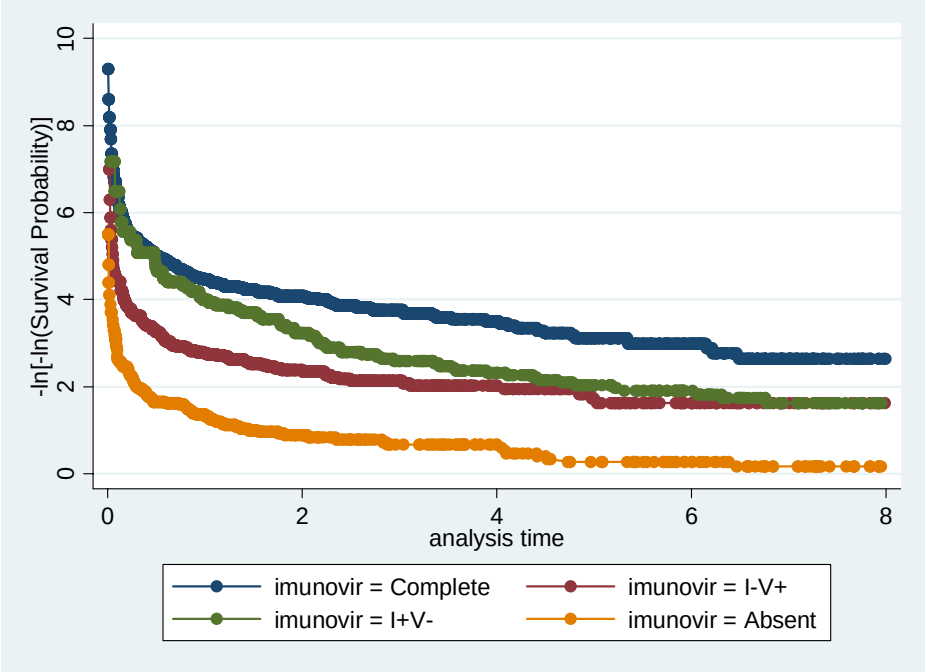
b) Baseline CD4 cell count (100 cells increase)



c) AIDS defining event



APPENDIX F. Log-log survival plots for categories of immunologic and virologic responses, adjusted for age, baseline CD4 count, site and AIDS defining events.



BIBLIOGRAPHY

1. Hogg, R.S., K.V. Heath, B. Yip, et al. (1998). Improved survival among HIV-infected individuals following initiation of antiretroviral therapy. *Jama*. **279**(6): p. 450-4.
2. Palella, F.J., Jr., K.M. Delaney, A.C. Moorman, et al. (1998). Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV Outpatient Study Investigators. *N Engl J Med*. **338**(13): p. 853-60.
3. Egger, M., B. Hirschel, P. Francioli, et al. (1997). Impact of new antiretroviral combination therapies in HIV infected patients in Switzerland: prospective multicentre study. Swiss HIV Cohort Study. *Bmj*. **315**(7117): p. 1194-9.
4. Mocroft, A., M. Youle, J. Morcinek, et al. (1997). Survival after diagnosis of AIDS: a prospective observational study of 2625 patients. Royal Free/Chelsea and Westminster Hospitals Collaborative Group. *Bmj*. **314**(7078): p. 409-13.
5. Hammer, S.M., K.E. Squires, M.D. Hughes, et al. (1997). 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. AIDS Clinical Trials Group 320 Study Team. *N Engl J Med*. **337**(11): p. 725-33.
6. Detels, R., A. Munoz, G. McFarlane, et al. (1998). Effectiveness of potent antiretroviral therapy on time to AIDS and death in men with known HIV infection duration. Multicenter AIDS Cohort Study Investigators. *Jama*. **280**(17): p. 1497-503.
7. Gulick, R.M., J.W. Mellors, D. Havlir, et al. (1997). Treatment with indinavir, zidovudine, and lamivudine in adults with human immunodeficiency virus infection and prior antiretroviral therapy. *N Engl J Med*. **337**(11): p. 734-9.
8. Montaner, J.S., P. Reiss, D. Cooper, et al. (1998). A randomized, double-blind trial comparing combinations of nevirapine, didanosine, and zidovudine for HIV-infected patients: the INCAS Trial. Italy, The Netherlands, Canada and Australia Study. *Jama*. **279**(12): p. 930-7.
9. Bartlett, J.A., R. DeMasi, J. Quinn, et al. (2001). Overview of the effectiveness of triple combination therapy in antiretroviral-naive HIV-1 infected adults. *Aids*. **15**(11): p. 1369-77.

10. Mocroft, A., M.J. Gill, W. Davidson, et al. (1998). Predictors of a viral response and subsequent virological treatment failure in patients with HIV starting a protease inhibitor. *Aids*. **12**(16): p. 2161-7.

11. Lucas, G.M., R.E. Chaisson and R.D. Moore (1999). Highly active antiretroviral therapy in a large urban clinic: risk factors for virologic failure and adverse drug reactions. *Ann Intern Med*. **131**(2): p. 81-7.

12. Ledergerber, B., M. Egger, M. Opravil, et al. (1999). Clinical progression and virological failure on highly active antiretroviral therapy in HIV-1 patients: a prospective cohort study. Swiss HIV Cohort Study. *Lancet*. **353**(9156): p. 863-8.

13. Staszewski, S., V. Miller, C. Sabin, et al. (1999). Virological response to protease inhibitor therapy in an HIV clinic cohort. *Aids*. **13**(3): p. 367-73.

14. Paredes, R., A. Mocroft, O. Kirk, et al. (2000). Predictors of virological success and ensuing failure in HIV-positive patients starting highly active antiretroviral therapy in Europe: results from the EuroSIDA study. *Arch Intern Med*. **160**(8): p. 1123-32.

15. Grabar, S., V. Le Moing, C. Goujard, et al. (2000). Clinical outcome of patients with HIV-1 infection according to immunologic and virologic response after 6 months of highly active antiretroviral therapy. *Ann Intern Med*. **133**(6): p. 401-10.

16. Le Moing, V., G. Chene, M.P. Carrieri, et al. (2002). Predictors of virological rebound in HIV-1-infected patients initiating a protease inhibitor-containing regimen. *Aids*. **16**(1): p. 21-9.

17. Pulido, F., J.R. Arribas, J.M. Miro, et al. (2004). Clinical, virologic, and immunologic response to efavirenz-or protease inhibitor-based highly active antiretroviral therapy in a cohort of antiretroviral-naive patients with advanced HIV infection (EfaVIP 2 study). *J Acquir Immune Defic Syndr*. **35**(4): p. 343-50.

18. Anastos, K., Y. Barron, M.H. Cohen, et al. (2004). The prognostic importance of changes in CD4+ cell count and HIV-1 RNA level in women after initiating highly active antiretroviral therapy. *Ann Intern Med*. **140**(4): p. 256-64.

19. Piketty, C., P. Castiel, L. Belec, et al. (1998). Discrepant responses to triple combination antiretroviral therapy in advanced HIV disease. *Aids*. **12**(7): p. 745-50.

20. Nicastri, E., A. Chiesi, C. Angeletti, et al. (2005). Clinical outcome after 4 years follow-up of HIV-seropositive subjects with incomplete virologic or immunologic response to HAART. *J Med Virol*. **76**(2): p. 153-60.

21. Mehta, S.H., G. Lucas, J. Astemborski, et al. (2007). Early immunologic and virologic responses to highly active antiretroviral therapy and subsequent disease progression among HIV-infected injection drug users. *AIDS Care*. **19**(5): p. 637-45.

22. Moore, D.M., R.S. Hogg, B. Yip, et al. (2005). Discordant immunologic and virologic responses to highly active antiretroviral therapy are associated with increased mortality and poor adherence to therapy. *J Acquir Immune Defic Syndr*. **40**(3): p. 288-93.

23. Schechter, M., M. Brinkhof, E. M., et al. Discordant Immunologic and Virologic Responses to ART among Previously Naive Adults Initiating HAART in Resource-constrained Settings. in *Thirteenth Conference on Retroviruses and Opportunistic Infections*. 2006. Denver, Abstract 559.

24. Kaufmann, D., G. Pantaleo, P. Sudre, et al. (1998). CD4-cell count in HIV-1-infected individuals remaining viraemic with highly active antiretroviral therapy (HAART). Swiss HIV Cohort Study. *Lancet*. **351**(9104): p. 723-4.

25. Piketty, C., L. Weiss, F. Thomas, et al. (2001). Long-term clinical outcome of human immunodeficiency virus-infected patients with discordant immunologic and virologic responses to a protease inhibitor-containing regimen. *J Infect Dis*. **183**(9): p. 1328-35.

26. Marimoutou, C., G. Chene, P. Mercie, et al. (2001). Prognostic factors of combined viral load and CD4+ cell count responses under triple antiretroviral therapy, Aquitaine cohort, 1996-1998. *J Acquir Immune Defic Syndr*. **27**(2): p. 161-7.

27. Teixeira, L., H. Valdez, J.M. McCune, et al. (2001). Poor CD4 T cell restoration after suppression of HIV-1 replication may reflect lower thymic function. *Aids*. **15**(14): p. 1749-56.

28. Brenchley, J.M., T.W. Schacker, L.E. Ruff, et al. (2004). CD4+ T cell depletion during all stages of HIV disease occurs predominantly in the gastrointestinal tract. *J Exp Med*. **200**(6): p. 749-59.

29. Douek, D.C., L.J. Picker and R.A. Koup (2003). T cell dynamics in HIV-1 infection. *Annu Rev Immunol*. **21**: p. 265-304.

30. Renaud, M., C. Katlama, A. Mallet, et al. (1999). Determinants of paradoxical CD4 cell reconstitution after protease inhibitor-containing antiretroviral regimen. *Aids*. **13**(6): p. 669-76.

31. Kim, R.B., M.F. Fromm, C. Wandel, et al. (1998). The drug transporter P-glycoprotein limits oral absorption and brain entry of HIV-1 protease inhibitors. *J Clin Invest*. **101**(2): p. 289-94.

32. Fellay, J., C. Marzolini, E.R. Meaden, et al. (2002). Response to antiretroviral treatment in HIV-1-infected individuals with allelic variants of the multidrug resistance transporter 1: a pharmacogenetics study. *Lancet*. **359**(9300): p. 30-6.

33. Brumme, Z.L., W.W. Dong, K.J. Chan, et al. (2003). Influence of polymorphisms within the CX3CR1 and MDR-1 genes on initial antiretroviral therapy response. *Aids*. **17**(2): p. 201-8.

34. Nasi, M., V. Borghi, M. Pinti, et al. (2003). MDR1 C3435T genetic polymorphism does not influence the response to antiretroviral therapy in drug-naive HIV-positive patients. *Aids*. **17**(11): p. 1696-8.

35. Huang, Y., W.A. Paxton, S.M. Wolinsky, et al. (1996). The role of a mutant CCR5 allele in HIV-1 transmission and disease progression. *Nat Med*. **2**(11): p. 1240-3.

36. Dean, M., M. Carrington, C. Winkler, et al. (1996). Genetic restriction of HIV-1 infection and progression to AIDS by a deletion allele of the CKR5 structural gene. Hemophilia Growth and Development Study, Multicenter AIDS Cohort Study, Multicenter Hemophilia Cohort Study, San Francisco City Cohort, ALIVE Study. *Science*. **273**(5283): p. 1856-62.

37. Brumme, Z.L., K.J. Chan, W. Dong, et al. (2001). CCR5Delta32 and promoter polymorphisms are not correlated with initial virological or immunological treatment response. *Aids*. **15**(17): p. 2259-66.

38. Fernandez, S., A.A. Rosenow, I.R. James, et al. (2006). Recovery of CD4+ T Cells in HIV patients with a stable virologic response to antiretroviral therapy is associated with polymorphisms of interleukin-6 and central major histocompatibility complex genes. *J Acquir Immune Defic Syndr*. **41**(1): p. 1-5.

39. Nasi, M., M. Pinti, R. Bugarini, et al. (2005). Genetic polymorphisms of Fas (CD95) and Fas ligand (CD178) influence the rise in CD4+ T cell count after antiretroviral therapy in drug-naive HIV-positive patients. *Immunogenetics*. **57**(9): p. 628-35.

40. Berry, N., K. Ariyoshi, O. Jobe, et al. (1994). HIV type 2 proviral load measured by quantitative polymerase chain reaction correlates with CD4+ lymphopenia in HIV type 2-infected individuals. *AIDS Res Hum Retroviruses*. **10**(8): p. 1031-7.

41. Negredo, E., J. Molto, D. Burger, et al. (2004). Unexpected CD4 cell count decline in patients receiving didanosine and tenofovir-based regimens despite undetectable viral load. *Aids*. **18**(3): p. 459-63.

42. Barreiro, P. and V. Soriano (2006). Suboptimal CD4 gains in HIV-infected patients receiving didanosine plus tenofovir. *J Antimicrob Chemother*. **57**(5): p. 806-9.

43. Barrios, A., A. Rendon, E. Negredo, et al. (2005). Paradoxical CD4+ T-cell decline in HIV-infected patients with complete virus suppression taking tenofovir and didanosine. *Aids*. **19**(6): p. 569-75.

44. Wood, E., R.S. Hogg, B. Yip, et al. (2002). "Discordant" increases in CD4 cell count relative to plasma viral load in a closely followed cohort of patients initiating antiretroviral therapy. *J Acquir Immune Defic Syndr*. **30**(2): p. 159-66.

45. Deeks, S.G., T. Wrin, T. Liegler, et al. (2001). Virologic and immunologic consequences of discontinuing combination antiretroviral-drug therapy in HIV-infected patients with detectable viremia. *N Engl J Med*. **344**(7): p. 472-80.

46. Sufka, S.A., G. Ferrari, V.E. Gryszowka, et al. (2003). Prolonged CD4+ cell/virus load discordance during treatment with protease inhibitor-based highly active antiretroviral therapy: immune response and viral control. *J Infect Dis*. **187**(7): p. 1027-37.

47. Kaplan, S.S., G. Ferrari, T. Wrin, et al. (2005). Longitudinal assessment of immune response and viral characteristics in HIV-infected patients with prolonged CD4(+)/viral load discordance. *AIDS Res Hum Retroviruses*. **21**(1): p. 13-6.

48. Deeks, S.G., R. Hoh, R.M. Grant, et al. (2002). CD4+ T cell kinetics and activation in human immunodeficiency virus-infected patients who remain viremic despite long-term treatment with protease inhibitor-based therapy. *J Infect Dis*. **185**(3): p. 315-23.

49. D'Ettorre, G., G. Forcina, M. Andreotti, et al. (2002). Discordant response to antiretroviral therapy: HIV isolation, genotypic mutations, T-cell proliferation and cytokine production. *Aids*. **16**(14): p. 1877-85.

50. Nicastri, E., L. Sarmati, G. d'Ettorre, et al. (2003). High prevalence of M184 mutation among patients with viroimmunologic discordant responses to highly active antiretroviral therapy and outcomes after change of therapy guided by genotypic analysis. *J Clin Microbiol*. **41**(7): p. 3007-12.

51. Sarmati, L., M. Montano, L. Dori, et al. (2004). Discordant response to HAART: reduction of viremia and replicative capacity of HIV strains in patients after genotype guided change of therapy. *New Microbiol*. **27**(2 Suppl 1): p. 95-8.

52. Barbour, J.D., T. Wrin, R.M. Grant, et al. (2002). Evolution of phenotypic drug susceptibility and viral replication capacity during long-term virologic failure of protease inhibitor therapy in human immunodeficiency virus-infected adults. *J Virol*. **76**(21): p. 11104-12.

53. Linden, D., S. Sufka, G. Ferrari, et al. Viral and Immune Correlates of Discordant CD4/VL Responses to NNRTI-based HAART and Comparison to a Discordant Cohort Receiving Protease Inhibitor-Based HAART. in *Tenth Conference on Retroviruses and Opportunistic Infections*. 2003. Boston, Abstract 146.

54. Pires, A., J. Pido-Lopez, G. Moyle, et al. (2004). Enhanced T-cell maturation, differentiation and function in HIV-1-infected individuals after growth hormone and highly active antiretroviral therapy. *Antivir Ther*. **9**(1): p. 67-75.

55. WHO, Progress on global access to HIV antiretroviral therapy: a report on "3 by 5" and beyond, March 2006. 2006, World Health Organization: Geneva.

56. Zhou, J., N. Kumarasamy, R. Ditangco, et al. (2005). The TREAT Asia HIV Observational Database: baseline and retrospective data. *J Acquir Immune Defic Syndr*. **38**(2): p. 174-9.

57. Ivers, L.C., D. Kendrick and K. Doucette (2005). Efficacy of antiretroviral therapy programs in resource-poor settings: a meta-analysis of the published literature. *Clin Infect Dis*. **41**(2): p. 217-24.

58. Braitstein, P., M.W. Brinkhof, F. Dabis, et al. (2006). Mortality of HIV-1-infected patients in the first year of antiretroviral therapy: comparison between low-income and high-income countries. *Lancet*. **367**(9513): p. 817-24.

59. Jaffar, S., T. Govender, A. Garrib, et al. (2005). Antiretroviral treatment in resource-poor settings: public health research priorities. *Trop Med Int Health*. **10**(4): p. 295-9.

60. Egger, M., A. Boulle, M. Schechter, et al. (2005). Antiretroviral therapy in resource-poor settings: scaling up inequalities? *Int J Epidemiol*. **34**(3): p. 509-12.

61. Bennett, S., J.T. Boerma and R. Brugha (2006). Scaling up HIV/AIDS evaluation. *Lancet*. **367**(9504): p. 79-82.

62. Dabis, F., E. Balestre, P. Braitstein, et al. (2005). Cohort Profile: Antiretroviral Therapy in Lower Income Countries (ART-LINC): international collaboration of treatment cohorts. *Int J Epidemiol*. **34**(5): p. 979-86.

63. Mellors, J.W., A. Munoz, J.V. Giorgi, et al. (1997). Plasma viral load and CD4+ lymphocytes as prognostic markers of HIV-1 infection. *Ann Intern Med*. **126**(12): p. 946-54.

64. Marins, J.R., L.F. Jamal, S.Y. Chen, et al. (2003). Dramatic improvement in survival among adult Brazilian AIDS patients. *Aids*. **17**(11): p. 1675-82.

65. Galvao, J. (2002). Access to antiretroviral drugs in Brazil. *Lancet*. **360**(9348): p. 1862-5.

66. Teixeira, P.R., M.A. Vitoria and J. Barcarolo (2004). Antiretroviral treatment in resource-poor settings: the Brazilian experience. *Aids*. **18 Suppl 3**: p. S5-7.

67. Levi, G.C. and M.A. Vitoria (2002). Fighting against AIDS: the Brazilian experience. *Aids*. **16**(18): p. 2373-83.

68. May, S.B., P.F. Barroso, E.P. Nunes, et al. (2007). Effectiveness of highly active antiretroviral therapy using non-brand name drugs in Brazil. *Braz J Med Biol Res*. **40**(4): p. 551-5.

69. Chene, G., J.A. Sterne, M. May, et al. (2003). Prognostic importance of initial response in HIV-1 infected patients starting potent antiretroviral therapy: analysis of prospective studies. *Lancet*. **362**(9385): p. 679-86.

70. (1993). From the Centers for Disease Control and prevention. 1993 revised classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults. *Jama*. **269**(4): p. 460.

71. Yeni, P.G., S.M. Hammer, M.S. Hirsch, et al. (2004). Treatment for adult HIV infection: 2004 recommendations of the International AIDS Society-USA Panel. *Jama*. **292**(2): p. 251-65.

72. Matthews, G.V., C.A. Sabin, S. Mandalia, et al. (2002). Virological suppression at 6 months is related to choice of initial regimen in antiretroviral-naive patients: a cohort study. *Aids*. **16**(1): p. 53-61.

73. Phillips, A.N., C. Pradier, A. Lazzarin, et al. (2001). Viral load outcome of non-nucleoside reverse transcriptase inhibitor regimens for 2203 mainly antiretroviral-experienced patients. *Aids*. **15**(18): p. 2385-95.

74. Mocroft, A., A.N. Phillips, N. Friis-Moller, et al. (2002). Response to antiretroviral therapy among patients exposed to three classes of antiretrovirals: results from the EuroSIDA study. *Antivir Ther*. **7**(1): p. 21-30.

75. Parienti, J.J., V. Massari, D. Descamps, et al. (2004). Predictors of virologic failure and resistance in HIV-infected patients treated with nevirapine- or efavirenz-based antiretroviral therapy. *Clin Infect Dis*. **38**(9): p. 1311-6.

76. Ickovics, J.R., A. Cameron, R. Zackin, et al. (2002). Consequences and determinants of adherence to antiretroviral medication: results from Adult AIDS Clinical Trials Group protocol 370. *Antivir Ther*. **7**(3): p. 185-93.

77. Wagner, G.J. and J.G. Rabkin (2000). Measuring medication adherence: are missed doses reported more accurately then perfect adherence? *AIDS Care*. **12**(4): p. 405-8.

78. Walsh, J.C., S. Mandalia and B.G. Gazzard (2002). Responses to a 1 month self-report on adherence to antiretroviral therapy are consistent with electronic data and virological treatment outcome. *Aids*. **16**(2): p. 269-77.

79. Kimmerling, M., G. Wagner and B. Ghosh-Dastidar (2003). Factors associated with accurate self-reported adherence to HIV antiretrovirals. *Int J STD AIDS*. **14**(4): p. 281-4.

80. Hugen, P.W., N. Langebeek, D.M. Burger, et al. (2002). Assessment of adherence to HIV protease inhibitors: comparison and combination of various methods, including MEMS (electronic monitoring), patient and nurse report, and therapeutic drug monitoring. *J Acquir Immune Defic Syndr*. **30**(3): p. 324-34.

81. Kalichman, S.C., B. Ramachandran and S. Catz (1999). Adherence to combination antiretroviral therapies in HIV patients of low health literacy. *J Gen Intern Med*. **14**(5): p. 267-73.

82. Hofer, C.B., M. Schechter and L.H. Harrison (2004). Effectiveness of Antiretroviral Therapy Among Patients Who Attend Public HIV Clinics in Rio de Janeiro, Brazil. *J Acquir Immune Defic Syndr*. **36**(4): p. 967-971.

83. Valdez, H., S.F. Purvis, M.M. Lederman, et al. (1999). Association of the CCR5delta32 mutation with improved response to antiretroviral therapy. *Jama*. **282**(8): p. 734.

84. Kitahata, M.M., S.E. Van Rompaey, P.W. Dillingham, et al. (2003). Primary care delivery is associated with greater physician experience and improved survival among persons with AIDS. *J Gen Intern Med*. **18**(2): p. 95-103.

85. Delgado, J., K.V. Heath, B. Yip, et al. (2003). Highly active antiretroviral therapy: physician experience and enhanced adherence to prescription refill. *Antivir Ther*. **8**(5): p. 471-8.

86. Egger, M., M. May, G. Chene, et al. (2002). Prognosis of HIV-1-infected patients starting highly active antiretroviral therapy: a collaborative analysis of prospective studies. *Lancet*. **360**(9327): p. 119-29.

87. Grabar, S., V. Le Moing, C. Goujard, et al. (2005). Response to highly active antiretroviral therapy at 6 months and long-term disease progression in HIV-1 infection. *J Acquir Immune Defic Syndr*. **39**(3): p. 284-92.

88. Moore, D.M., R.S. Hogg, K. Chan, et al. (2006). Disease progression in patients with virological suppression in response to HAART is associated with the degree of immunological response. *Aids*. **20**(3): p. 371-7.

89. Deeks, S.G. and R.M. Grant (1999). Sustained CD4 responses after virological failure of protease inhibitor-containing therapy. *Antivir Ther*. **4 Suppl 3**: p. 7-11.

90. Mehta, S., Lucas G, Astemborski J, Kirk G, Vlahov D, Galai N. Discordant Responses to HAART and Clinical Outcomes among Injection Drug Users in Baltimore, Maryland. in *Thirteenth Conference on Retroviruses and Opportunistic Infections*. 2006. Denver, Abstract 527.

91. Tuboi, S.H., L.H. Harrison, E. Sprinz, et al. (2005). Predictors of virologic failure in HIV-1-infected patients starting highly active antiretroviral therapy in Porto Alegre, Brazil. *J Acquir Immune Defic Syndr*. **40**(3): p. 324-8.

92. Coetzee, D., K. Hildebrand, A. Boulle, et al. (2004). Outcomes after two years of providing antiretroviral treatment in Khayelitsha, South Africa. *Aids*. **18**(6): p. 887-95.

93. Landman, R., R. Schiemann, S. Thiam, et al. (2003). Once-a-day highly active antiretroviral therapy in treatment-naive HIV-1-infected adults in Senegal. *Aids*. **17**(7): p. 1017-22.

94. Holmes, C.B., E. Losina, R.P. Walensky, et al. (2003). Review of human immunodeficiency virus type 1-related opportunistic infections in sub-Saharan Africa. *Clin Infect Dis*. **36**(5): p. 652-62.

95. Rubin, D., Multiple imputation for nonresponse in surveys. 1987, New York: Wiley.

96. Glass, T.R., S. De Geest, R. Weber, et al. (2006). Correlates of self-reported nonadherence to antiretroviral therapy in HIV-infected patients: the Swiss HIV Cohort Study. *J Acquir Immune Defic Syndr*. **41**(3): p. 385-92.

97. Hinkin, C.H., D.J. Hardy, K.I. Mason, et al. (2004). Medication adherence in HIV-infected adults: effect of patient age, cognitive status, and substance abuse. *Aids*. **18 Suppl 1**: p. S19-25.

98. Spritzler, J., D. Mildvan, A. Russo, et al. (2003). Can immune markers predict subsequent discordance between immunologic and virologic responses to antiretroviral therapy? Adult AIDS Clinical Trials Group. *Clin Infect Dis*. **37**(4): p. 551-8.

99. Barreiro, P., V. Soriano, E. Casas, et al. (2002). Different degree of immune recovery using antiretroviral regimens with protease inhibitors or non-nucleosides. *Aids*. **16**(2): p. 245-9.

100. Benito, J.M., M. Lopez, J.C. Martin, et al. (2002). Differences in cellular activation and apoptosis in HIV-infected patients receiving protease inhibitors or nonnucleoside reverse transcriptase inhibitors. *AIDS Res Hum Retroviruses*. **18**(18): p. 1379-88.

101. Karrer, U., B. Ledergerber, H. Furrer, et al. (2005). Dose-dependent influence of didanosine on immune recovery in HIV-infected patients treated with tenofovir. *Aids*. **19**(17): p. 1987-94.

102. Wood, E., R.S. Hogg, K.V. Heath, et al. (2003). Provider bias in the selection of non-nucleoside reverse transcriptase inhibitor and protease inhibitor-based highly active antiretroviral therapy and HIV treatment outcomes in observational studies. *Aids*. **17**(18): p. 2629-34.

103. Acock, A.C. (2005). Working with missing values. *Journal of Marriage and Family*(67): p. 1012–1028.

104. Tuboi, S.H., M.W. Brinkhof, M. Egger, et al. (2007). Discordant responses to potent antiretroviral treatment in previously naive HIV-1-infected adults initiating treatment in resource-constrained countries: the antiretroviral therapy in low-income countries (ART-LINC) collaboration. *J Acquir Immune Defic Syndr*. **45**(1): p. 52-9.

105. Hogg, R.S., D.R. Bangsberg, V.D. Lima, et al. (2006). Emergence of drug resistance is associated with an increased risk of death among patients first starting HAART. *PLoS Med*. **3**(9): p. e356.

106. Moore, R.D. and J.C. Keruly (2007). CD4+ cell count 6 years after commencement of highly active antiretroviral therapy in persons with sustained virologic suppression. *Clin Infect Dis*. **44**(3): p. 441-6.

107. Badri, M., S.D. Lawn and R. Wood (2006). Short-term risk of AIDS or death in people infected with HIV-1 before antiretroviral therapy in South Africa: a longitudinal study. *Lancet*. **368**(9543): p. 1254-9.

108. Moh, R., C. Danel, E. Messou, et al. (2007). Incidence and determinants of mortality and morbidity following early antiretroviral therapy initiation in HIV-infected adults in West Africa. *Aids*. **21**(18): p. 2483-2491.

109. Wood, E., R.S. Hogg, B. Yip, et al. (2003). Effect of medication adherence on survival of HIV-infected adults who start highly active antiretroviral therapy when the CD4+ cell count is 0.200 to 0.350 x 10(9) cells/L. *Ann Intern Med*. **139**(10): p. 810-6.

110. Hogg, R.S., K. Heath, D. Bangsberg, et al. (2002). Intermittent use of triple-combination therapy is predictive of mortality at baseline and after 1 year of follow-up. *Aids*. **16**(7): p. 1051-8.

111. Hunt, P.W., S.G. Deeks, D.R. Bangsberg, et al. (2006). The independent effect of drug resistance on T cell activation in HIV infection. *Aids*. **20**(5): p. 691-9.

112. Anthony, K.B., C. Yoder, J.A. Metcalf, et al. (2003). Incomplete CD4 T cell recovery in HIV-1 infection after 12 months of highly active antiretroviral therapy is associated with ongoing increased CD4 T cell activation and turnover. *J Acquir Immune Defic Syndr*. **33**(2): p. 125-33.

113. Schechter, M. (2007). Treatment at scale in Brazil: a physician's perspective. *Aids*. **21 Suppl 4**: p. S31-5.

114. Greco, D.B. and M. Simao (2007). Brazilian policy of universal access to AIDS treatment: sustainability challenges and perspectives. *Aids*. **21 Suppl 4**: p. S37-45.

115. Nunn, A.S., E.M. Fonseca, F.I. Bastos, et al. (2007). Evolution of antiretroviral drug costs in Brazil in the context of free and universal access to AIDS treatment. *PLoS Med*. **4**(11): p. e305.

116. Barnett, A.G., J.C. van der Pols and A.J. Dobson, Regression to the mean: what it is and how to deal with it. 2005. p. 215-220.