Association of HLA profiles with early plasma viral load, CD4+ cell count and rate of progression to AIDS following acute HIV-1 infection

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Background: Host genetic factors, such as HLA alleles, play an important role in mediating the course of HIV-1 disease progression through largely undefined mechanisms.

Objectives: To examine the association of HLA markers with HIV-1 RNA plasma viral load and other factors associated with course of disease progression in HIV-1 infection.

Design and methods: A group of 139 HIV-1 seroconverters from the Multicenter AIDS Cohort Study had been typed for a variety of HLA markers. HIV-1 RNA plasma viral load was measured from frozen plasma specimens obtained approximately 9 months following seroconversion. CD4+ cell counts were available from the same study visit. Statistical analysis was performed using survival techniques and linear regression models to quantify the relative associations of an HLA score profile, HIV-1 RNA plasma viral load, CD4+ cell count and age with each other and with rate of progression to AIDS and death.

Results: Cox proportional hazards models showed statistically significant differences in time to AIDS by HLA score profile category per unit increase [relative hazard (RH), 0.64; $P < 0.0001$], HIV-1 RNA plasma viral load per 10-fold increase (RH, 2.04; $P = 0.0003$), and CD4+ cell count per 100 cell ($\times 10^6$/l) increase (RH, 0.90; $P = 0.02$). Multivariate linear regression showed that viral load was 39% lower ($P = 0.0001$) for each unit increase in HLA score profile and 13% lower ($P = 0.002$) for each 100 cell ($\times 10^6$/l) increase in CD4+ cell count.

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Conclusions: The means by which the HLA score profile influences the time to AIDS is probably through immunologic responses that affect the rate of HIV-1 replication, as manifested by the HIV-1 RNA plasma viral load during the first 6–12 months following acute infection.

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Introduction

HIV-1 causes progressive immune deterioration and ultimately clinically manifests as AIDS in humans. But as with all etiologic agents, HIV must replicate in the milieu of a given host to produce disease, and it is the balance between the organism's virulence and the resistance of the host that determines the course and severity of disease. Accordingly, certain intrinsic host characteristics, such as older age and genetic makeup, may be true risk factors for rate of progression to AIDS or death. Other markers that change quickly after infection, such as CD4+ cell count, neopterin and β_2-microglobulin levels, are consequences rather than determinants of disease progression. Early HIV-1 plasma viral load is both a consequence of events at inception of the infection and closely related causally to the rate of future disease progression [1]. The number of viral particles in plasma is proportional to the total number of productively infected CD4+ lymphocytes in a given person [2], which in turn is related to the ability of the particular host to contain the infection. Recent reports [2–4] on viral dynamics and the relationship between viral load and rate of progression [1] also show that plasma viral load is in the causal pathway for AIDS, leading to the quantitative loss of CD4+ lymphocytes. In addition, estimating the rate of progression of HIV-1 infection is most reliably accomplished by measuring the incubation time from HIV-1 seroconversion to AIDS or death.

Although replication of HIV-1 produces disease, host factors appear to strongly influence these rates of replication in different persons, in that they account for a wide range of incubation periods observed for AIDS [5]. Kaslow *et al.* [6] showed that combinations of markers in the HLA region predict time to AIDS, and Pantaleo *et al.* [7] have focused on host–viral interaction during very early HIV-1 infection as a prime determinant of subsequent course of disease progression. Fig. 1 summarizes a likely sequence in the pathogenetic order of risk variables and outcomes that prompted this study. In this report, we corroborate that HLA profile is highly associated with rate of progression and suggest that the products of these genes exert

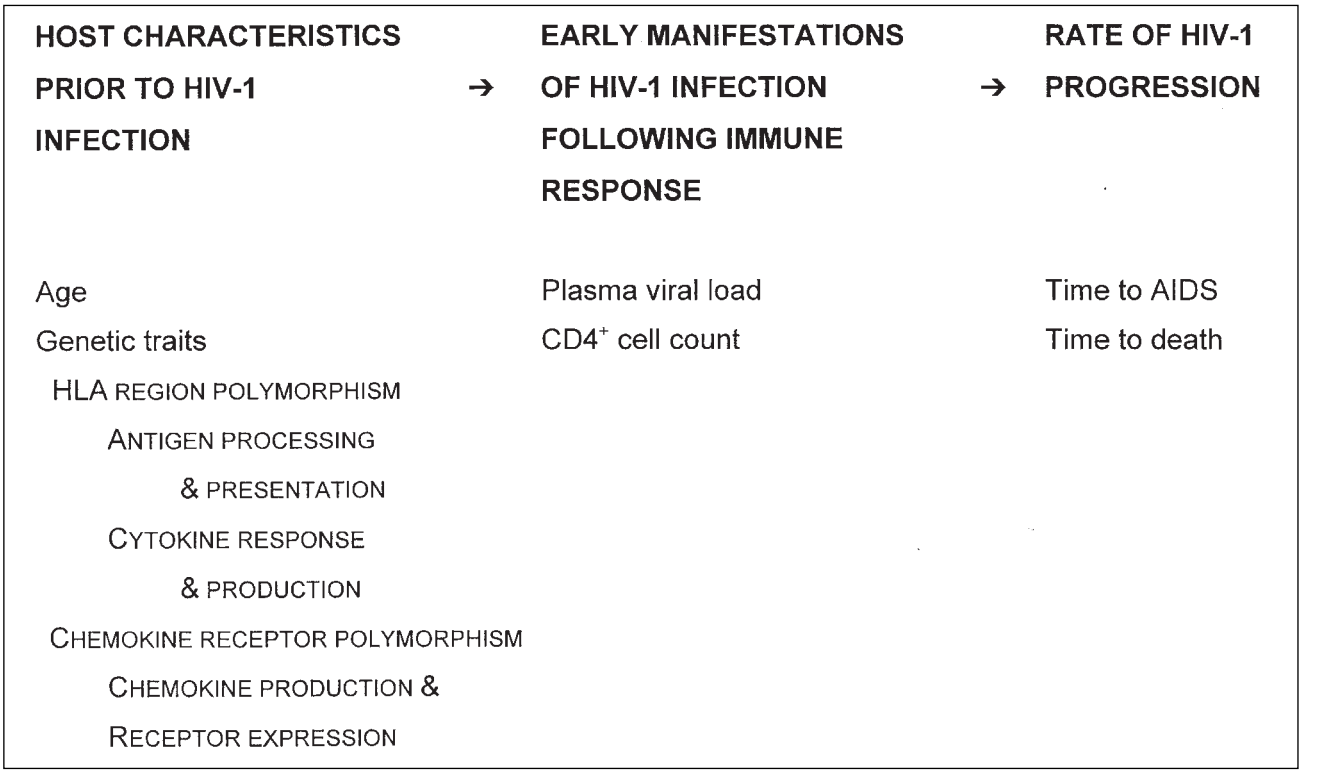

Fig. 1. Host characteristics, risk factors and outcomes in the causal pathway of HIV-1 disease progression.

their effect early in HIV-1 infection through the pathway that establishes the virus concentration (i.e., plasma viral load). This identifies at least one mechanism whereby events within 6–12 months following seroconversion during HIV-1 replication determine the rate of progression.

Methods

Study population

The Multicenter AIDS Cohort Study (MACS), initiated in 1984, longitudinally evaluates the natural history of HIV-1 infection in homosexual men [8]. Occurrence of AIDS and death in this cohort are continuously updated. AIDS is defined here by the 1987 surveillance definition [9]. Participants are monitored for HIV infection and CD4 cell count every 6 months. Of the 3141 initially HIV-seronegative entrants, 487 seroconverted prior to May 1996. Of these 487 participants, 139 white men were selected for HLA typing on the basis of rate of disease progression and length of follow-up. By 17 November 1993, 71 men had developed AIDS and 68 had not. For this analysis, CD4+ cell count and plasma viral load were measured a median of 9 months (interquartile range, 7.9–9.7 months) after seroconversion, the midpoint between the last seronegative and first seropositive visit.

Kaslow *et al.* [6] identified combinations of HLA markers in these 139 participants that conferred either protective or adverse effects on the rate of HIV disease progression. An HLA scoring profile was developed by means of a detailed analysis using Cox proportional hazards models [10] to compute the relative hazards of AIDS following HIV-1 seroconversion in men carrying a given marker compared with all men not carrying that marker. The HLA scoring profile effectively discriminated 71 AIDS cases from the remaining 68 participants who were AIDS-free as of 17 November 1993. Table 1 shows the value (−1, 0, or +1) assigned to each marker combination. For each individual who carried any of those markers shown, HLA score profile was calculated as the algebraic sum of the values given to those markers. The predictive capacity of the score profile was independently confirmed in two different cohorts of homosexual men [6,11].

This analysis extends the follow-up of 139 participants to 21 February 1996. Taking advantage of the additional follow-up time, from 13 November 1993 to 21 February 1996, we corroborated the ability of the HLA scoring profile to predict subsequent AIDS amongst the 68 participants, referred to as the confirming subset, who were AIDS-free at the time of the initial analysis when the scoring profile was developed. Twenty-six of the 68 persons developed AIDS during the extended follow-up time period; the capability of the HLA scoring profile to discriminate these 26 AIDS cases from the other 42 would, therefore, not be vulnerable to a potential circular bias.

Table 1. HLA markers and combinations that are algebraically summed to give the HLA score profile.

Markers
Counted as +1 (protective effect)
B27
B51
B57
A25 and TAP2.3
A26 and TAP2.3
A32 and TAP2.3
B18 and TAP2.3
Counted as −1 (adverse effect)
B37
B49
A28 and TAP2.3
A29 and TAP2.1
B8 and TAP2.1
A23 and not TAP2.3
A24 and (TAP2.1 or TAP2.3)
B60 and (TAP2.1 or TAP2.3)
B35 and C4
DRB1*0401–DQA1*0300–DQB1*0301 and TAP1.2
DRB1*1200–DQA1*0501–DBQ1*0301 and TAP1.2
DRB1*1300–DQA1*0102–DBQ1*0604 and TAP1.2
DRB1*1400–DQA1*0101–DBQ1*0503 and TAP1.2

Laboratory methods

As described previously [6], class I typing was performed serologically by conventional microcytotoxicity assay and reagents. Molecular amplification methods were used on lymphocyte DNA for class II alleles (DRB1, DQA1, DQB1) and for polymorphic segments in the genes encoding transporter proteins associated with antigen processing (TAP1 and TAP2). Lymphocytes were phenotypically analyzed by two-color flow cytometry of either stained specimens of Ficoll-Hypaque-separated peripheral blood mononuclear cells or EDTA-anticoagulated whole blood stained with fluorescein- and phycoerythrin-conjugated antibodies, as previously described [12,13]. HIV-1 RNA (plasma viral load) was quantified in heparinized plasma samples stored at 70°C. A sensitive (version 2) branched DNA assay (Chiron Corporation, Emeryville, California, USA) was used, which has a lower quantification limit of 500 HIV-1 RNA copies/ml [14]. Samples were tested from duplicate 1.0 ml specimens after viral particles were pelleted by centrifugation at 23 500 **g** for 1 h at 4°C.

Statistical analysis

Cox proportional hazard models were fit to risk variables with time to AIDS and duration of survival from incident HIV-1 infection as outcomes [10]. Such variables included CD4+ lymphocyte count at the time HIV-1 viral load was measured, age at HIV-1 seroconversion and HLA scoring profile in five categories

($\leq -2, -1, 0, +1, \geq +2$), which represent the algebraic sum of positive and negative integers assigned to HLA markers associated with slower and more rapid progression of infection, respectively [6]. For numerical stability, only three HLA scoring profile categories (< 0, n = 50; 0, n = 54; > 0, n = 35) were summarized in the Kaplan–Meier plots. In order to maintain comparability of the Kaplan–Meier plots, the CD4+ cell count and HIV-1 RNA viral load data were trichotomized into similarly sized groups as these three HLA scoring profile groups.

Linear regression was used to study the association of HLA scoring profile with viral load and CD4+ cell count, the association of viral load with CD4+ cell count, and the association of CD4+ cell count with viral load. Viral load data were $\log_{10}$-transformed. CD4+ cell count was analyzed in increments of 100 cells ($\times 10^6$/l).

In order to reassure ourselves that the observed associations did not simply reflect HLA–AIDS relationships that were purposefully maximized by our original analytic strategy [6], analyses were replicated both excluding and including the 71 cases of AIDS that occurred before 1994 on whose discrimination the HLA scoring profile was originally constructed.

Results

The group of 139 men who were HLA typed were representative of all seroconverters as of 1995 in important risk factors for progression (Table 2). The confirming subset who remained AIDS-free until 1995 (n = 68), of whom 26 subsequently developed AIDS, had higher HLA scoring profiles, higher CD4+ cell counts, and lower viral load than those who developed AIDS earlier. The overall median follow-up of the 139 participants was 9.5 years and for the confirming subset of 68 was 10.7 years. Table 3 compares the 71 AIDS cases who were used to develop the HLA scoring profile with the 26 who subsequently developed AIDS and with the 42 who remained AIDS-free. The expected trend of baseline and early markers of infection was observed with the original 71 AIDS cases having the worst values and the 42 AIDS-free having the best. The HLA scoring profile was significantly lower in the 26 subsequent AIDS cases than in the 42 AIDS-free group ($P = 0.05$), reconfirming that the HLA scoring profile has predictive value for AIDS that occurred after its initial development.

Stratified Kaplan–Meier graphs (Fig. 2) show the relationships of the HLA scoring profile, HIV-1 RNA plasma viral load and CD4+ cell count to time to AIDS in the 139 seroconverters. Median times to AIDS were 5.2, 7.8 and 10.7 years for the three HLA scoring profile levels (< 0, 0 and > 0; Fig. 2A). The median times to AIDS in the graphs for HIV-1 RNA categories (Fig. 2B) and CD4+ cell count categories (Fig. 2C) were virtually identical to those of the HLA scoring profile. The median times to death by HLA scoring profile score category were 6.9, 9.8 and 11.4 years.

Cox proportional hazard models for the entire 139 participants are shown in Table 4 for risk of AIDS. HLA scoring profile, but not age, was significantly associated with the time to AIDS in both univariate and multivariate analyses. The relative hazard of AIDS for each incremental change in HLA scoring profile was 0.64 in the multivariate model, which included age. A propor-

Table 2. Means (SD) of variables in all Multicenter AIDS Cohort Study (MACS) seroconverters, those seroconverters who were HLA-typed and those who had not developed AIDS when the HLA score profile was developed.

Variable	All MACS seroconverters (n = 487)	HLA group (n = 139)	Confirming subset (n = 68)
Host factors			
Age at seroconversion (years)	34.6 (8.0)	34.0 (8.1)	33.1 (8.4)
HLA score profile	NA	2.8 (1.1)	3.4 (0.9)
Post-seroconversion factors			
Early viral load (copies/ml)*	NA	11023 (19841)	4953 (8915)
Early CD4 cells ($\times 10^6$/l)*	655 (266)	601 (361)	735 (367)

*Geometric means obtained a median of 9 months after seroconversion. NA, Not applicable.

Table 3. Data demonstrating the consistency of the relationship of the HLA scoring profile as reflected in plasma HIV RNA viral load and CD4+ cell count in individuals who had follow-up after the HLA scoring profile was developed.

	n	HLA scoring profile	Geometric mean	
			Plasma HIV RNA (copies/ml)	CD4 cell count ($\times 10^6$/l)
First cases of AIDS*	71	2.24	21900	512
Subsequent AIDS†	26	3.08	9300	656
AIDS-free	42	3.60	3300	802

*Occurred before the HLA scoring profile was developed. †Occurred after the HLA scoring profile was developed.

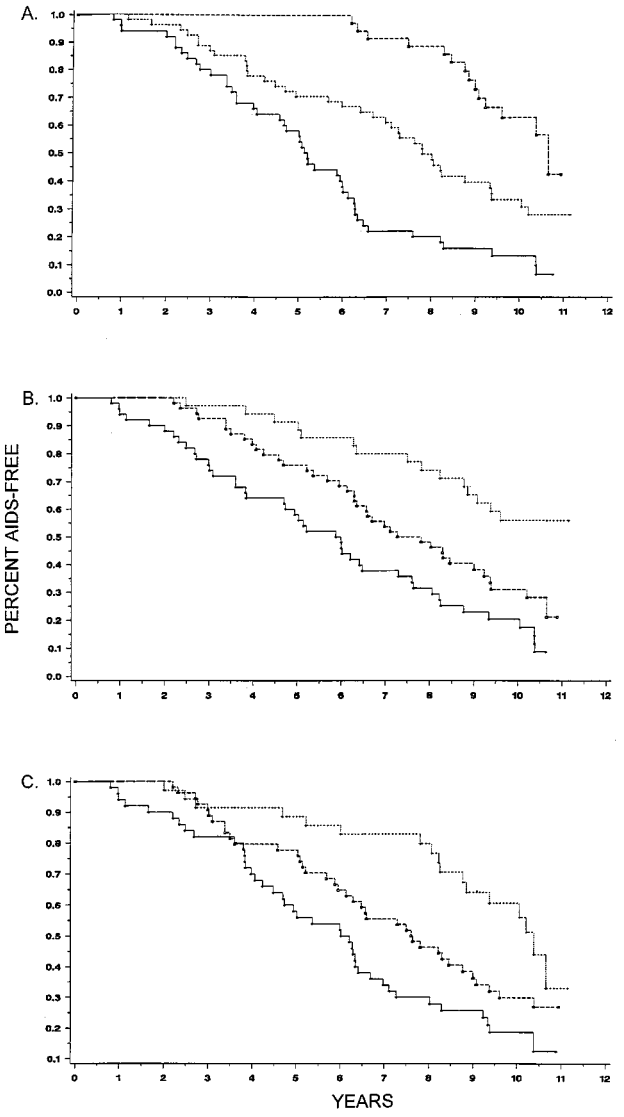

Fig. 2. (A) Kaplan–Meier curves showing time from seroconversion to AIDS by the three HLA scoring profile categories (< 0, solid line, n = 50; 0, middle dashed line, n = 54; and > 0, upper dashed line, n = 35). This plot extends the data of the original published plot by approximately 2 years [6] ($\chi^2 = 39.6$, $P = 0.0001$). (B) Plot shows the time from seroconversion to AIDS by HIV-1 RNA levels obtained a median of 9 months following seroconversion. The groupings for HIV-1 RNA (n = 50, solid line, range 24 870–588 400 copies/ml; n = 54, middle dashed line, range 4099–24 750 copies/ml; n = 35, upper dashed line, range < 500–3745 copies/ml) intentionally matches the same relative numbers in each group as HLA scoring profile score category ($\chi^2 = 22.8$, $P = 0.0001$). (C) Plot shows the time from seroconversion to AIDS by CD4 cell count at the time of the viral load determination. The groupings for CD4+ cell count (n = 50, solid line, range 26–506 $\times 10^6$/l; n = 54, middle dashed line, range 508–847 $\times 10^6$/l; n = 35, upper dashed line, range 854–1915 $\times 10^6$/l) intentionally matches the same relative numbers in each group as HLA scoring profile score category ($\chi^2 = 15.5$, $P = 0.0004$).

tional hazards model was also fit using the subset of 68 who were AIDS-free at the time the HLA scoring profile was developed. Within this subset, the relative hazard for time to AIDS per unit change (e.g., −1 to 0) in HLA scoring profile was 0.69 ($P = 0.05$) in the univariate model, only somewhat higher than the relative hazard of 0.52 for the entire group of 139. Early post-seroconversion risk factors, plasma viral load and CD4+ cell count, were both significantly associated with time to AIDS. The impact of these variables on time to death was similar (data not shown).

In both univariate and multivariate linear regression models (Table 5), the HLA scoring profile and CD4+ cell count, but not age, were statistically associated with HIV-1 RNA plasma viral load. Similar findings were made using non-parametric Spearman correlations. Table 5 shows in the multivariate model that for each unit increment in the HLA scoring profile the plasma viral load was 39% lower. There was a significant association between CD4+ cell count and plasma viral load. For every 100 cell ($\times 10^6$/l) increment in CD4+ cell count, viral load was 13% lower after controlling for age and HLA scoring profile. HLA scoring profile, plasma viral load and age were also studied in univariate and multivariate linear regression models where CD4+ cell count, in increments of 100, was the dependent variable. For every unit increase in the HLA scoring profile in a multivariate model, the CD4+ cell count was higher by 58×10^6 cells/l (0.58 of the 100 cell increment; $P = 0.03$). For each $\log_{10}$ increase in the plasma viral load, the CD4+ cell count was lower by 116×10^6 cells/l (1.16×100 cell increment; $P = 0.002$). However, age had no effect on early CD4+ cell count.

Discussion

HIV-1 replication is clearly an important determinant of disease progression in HIV-1 infection [1]. Its strength as a predictor of subsequent disease progression probably derives from the CD4+ lymphocyte infection burden in a given host [2–4]. It is therefore instructive to consider the host factors that may be involved in determining the HIV-1 RNA viral load level shortly after HIV-1 seroconversion. Fig. 1 depicts a causal pathway where immunogenetic makeup, which dictates the immune response to HIV-1 through a number of potential mechanisms, is a primary factor. Correlations between HLA scoring profile, early plasma viral load, CD4+ cell count and time to AIDS or death support both an underlying genetic influence on HIV disease progression and an effect that takes place relatively soon after seroconversion. Such genetically mediated mechanisms controlling HIV-1 replication probably include antigen processing characteristics,

Table 4. Cox models showing the relative importance of pre- and post-seroconversion factors on the relative hazard (RH) of AIDS.

Variable	Univariate RH	*P*	Multivariate RH	*P*
Host factors				
HLA score profile (per score unit*)	0.52	< 0.0001	0.64	< 0.0001
Age at seroconversion† (per decade)	1.12	NS	1.16	NS
Post-seroconversion factors				
Early viral load‡ (per 10-fold increase)	2.66	< 0.0001	2.04	0.0003
Early CD4 cell count‡ (per 100×10^6 cell/l increase)	0.84	< 0.0001	0.90	0.02

*For example, 0 to +1. †Age at seroconversion was approximately 9 months younger than age at viral load testing for each individual; results are identical if age at viral load testing is used. ‡Median of 9 months after seroconversion to HIV-1.

Table 5. Associations between $\log_{10}$ plasma HIV-1 RNA viral load per unit difference in the predictor variables*.

Variable	Univariate model (%)	*P*	Multivariate model (%)	*P*
Age (per decade)	+12	0.54	+1	0.94
HLA score profile (per score unit)	–45	0.0001	–39	0.0001
CD4+ cell count (per 100×10^6 cells/l)	–17	0.0001	–13	0.002

*Obtained from linear regression analysis on $\log_{10}$-transformed plasma HIV-1 RNA viral load.

β-chemokine production, expression and phenotype of chemokine receptors and endogenous cytokine production, amongst others [15–17]. The specific sequence of immunologic events through which the HLA genes represented in the HLA scoring profile act are still unclear and need further study. In addition, we have not considered HIV-1-specific factors related to either virulence or attenuation that may independently contribute to the rate of disease progression.

HLA scoring profile and viral load were stronger predictors of time to AIDS by the Wald χ^2 statistic (Table 4) than CD4+ cell count, all of which were also independently significantly associated with time to AIDS. The strong associations of HLA scoring profile with viral load and CD4 cell counts at approximately 9 months after seroconversion may provide useful quantitative relationships (Table 5). For instance, multivariate linear regression allows for quantitative associations to be made between HLA scoring profile and viral load and also on the subsequent associations with CD4+ cell count. We noted that for every HLA scoring profile unit increase, the viral load was 0.39 lower than the value of HLA scoring profile immediately preceding it. These relationships may ultimately lead to understanding the relative contributions of host factors that affect HIV-1 replication and viral load, especially when specific allele combinations or chemokine receptor expression are found to be responsible for the bulk of the difference in viral load. The mechanism by which HLA scoring profile may mediate its effect is probably through cytotoxic T-lymphocyte and natural killer cellular responses [18,19]. Early data indicate that the chemokine receptor/ligand systems (encoded on different chromosomes and operating through a different mechanism) influences viral load and outcomes independently of HLA effects [20].

Age is clearly associated with shorter duration in time to AIDS in many populations, including the MACS [6,21], but in contrast to HLA scoring profile, age was not associated with early plasma viral load and CD4+ cell count in the MACS. While a type II error is possible, this finding suggests that the effect of age may not occur early after seroconversion, but may accumulate slowly over time or may be mediated through senescence of an immune system that is relatively unable to replenish itself, as opposed to a direct effect through early plasma viral load.

As previously noted by Kaslow *et al.* [6], some qualification of HLA scoring profile validity is needed because it was originally constructed in this cohort. However, this HLA scoring profile has been independently confirmed in other cohorts [6,11]. The validity of the HLA scoring profile is also demonstrated in Table 3, which shows that HLA scoring profile, viral load and CD4+ cell count maintained a relationship with each other that was consistent with their respective association with rate of progression to AIDS. In addition, HLA scoring profile has continued to predict onset of AIDS in this group beyond the original date of its construction both in Table 4 and in restricted analyses excluding the 71 AIDS cases occurring prior to 1994. The univariate relative hazard for the HLA scoring profile effect on AIDS was 0.69 in the confirming subset analysis, only somewhat higher than the relative hazard of 0.52 for the entire group of 139 participants. It is probably arguable that the HLA scoring profile had an 'advantage' over the other risk variables in the analysis of the entire group because of the way it was developed. However, the confirmatory subset of 68 participants is important because it supports the contention that circular bias did not play a significant role here and indicates the robust nature of the HLA scoring profile beyond the original separation of the 71 initial AIDS cases from the 68 others.

The HLA scoring profile predicted risk of HIV-1 disease progression dramatically and independently of

HIV-1 RNA viral load and CD4+ cell count. If these findings are confirmed, HLA alleles or a refined HLA scoring profile may become useful clinically to judge a patient's need for antiretroviral treatment. Such information would be used in conjunction with viral load and CD4+ cell count, particularly among patients with early stage HIV-1 infection. Identifying the highly relevant HLA markers would make this task simpler and reduce costs. Given that molecular amplification technologies are similar, we would expect the cost of measuring relevant HLA markers to be comparable to that of measuring of HIV-1 RNA plasma viral load, and measuring HLA markers would need to be performed only once.

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Appendix

The Multicenter AIDS Cohort Study

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