CD8-CD4 disequilibrium predicts the risk continuum of HIV acquisition, AIDS, and muted immunologic reconstitution despite early therapy

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Abstract

Many younger, HIV+ persons receiving virally suppressive antiretroviral therapy (ART) remain immunologically impaired and share certain disease risks in common with older, HIV− persons. Our results from HIV− (n=9,827) and HIV+ (n=6,336) cohorts suggest that this commonality relates to a distinct immunosuppression-mediating program – age-independent immunosenescence (AIIS) – activated in response to repeated antigenic stimuli associated with aging, risk factors for HIV, and HIV infection. AIIS and advanced AIIS indicate distinct grades of immunosuppression, quantifiable via novel CD8-CD4 equilibrium metrics that track degree of disequilibrium between CD8+ and CD4+ T-cell levels. AIIS may precede and contribute to the likelihood of HIV acquisition. Advanced AIIS, present in nearly 40% of patients during primary/early HIV infection, is genetically determined and predicts AIDS. The presence of advanced AIIS when starting ART predicts development of AIDS/non-AIDS comorbidities and constrained capacity for rapid reconstitution of CD8-CD4 equilibrium (AIIS-free status), regardless of when (relative to HIV acquisition) or at what CD4+ count ART is initiated. Reconstitution of AIIS-free status restores immunologic and transcriptomic attributes to levels found in AIIS-free, HIV− persons. Thus, AIIS is an inducible, quantifiable, and potentially reversible antigen-activated program that underpins the risk/severity continuum of HIV acquisition, severe immune depletion, and failure of optimal immune reconstitution, as well as the decline of immune function with age. This immunologic framework has actionable utility for precision HIV medicine: CD8-CD4 equilibrium metrics identify persons with a proclivity for this risk/severity continuum, providing the opportunity for early preventive and therapeutic interventions aimed at suppressing activation of the AIIS program.

INTRODUCTION

The ultimate goal of HIV medicine, short of cure, is restoration of immunologic integrity of HIV-1-seropositive (HIV+) persons to a level equivalent to that of age-matched, HIV-seronegative (HIV−) persons. This immunologic landmark is thought to be attainable by initiating virally suppressive antiretroviral therapy (ART) soon after infection or at higher CD4+ T-cell counts (*1*). However, our previous evaluation of large, well-characterized cohorts of persons who initiated ART during primary or early HIV infection revealed quandaries: (i) CD4+ T-cell counts in many HIV+ persons failed to normalize to levels typically found in HIV− persons (≥800 cells/mm^3), despite starting ART at relatively high CD4+ counts (≥500 cells/mm^3), and (ii) some persons developed AIDS-defining illnesses, despite normalization of CD4+ counts (*2, 3*). These quandaries suggest that (i) initiation of ART soon after HIV acquisition, even at higher CD4+ counts, is insufficient to attain the abovementioned immunologic landmark and (ii) CD4+ counts are an imprecise measure of immunologic integrity. Additionally, a substantial proportion of younger HIV+ persons receiving ART manifest diseases typically observed in older HIV− persons (e.g., cancer) (*4, 5*). In this study, we conceptualized a new framework of immunologic integrity and associated disease risks that may explain these quandaries and tested it in cohorts of HIV− (n=9,827) and HIV+ (n=6,336) participant samples.

The traditional concept is that lower immunologic integrity commences consequent to HIV acquisition from chronic antigenic stimulation by HIV viremia, which in turn, may be a proximate cause of CD4+ T-cell depletion (*6-8*). Immunologic integrity of HIV+ persons is benchmarked against that of age- and sex-matched HIV− persons with presumably intact

immunologic integrity. However, this benchmarking strategy may be imprecise, as all people experience repetitive antigenic stimuli throughout life (e.g., viral infections). Behavioral (e.g., unprotected sex with multiple partners, drug use) (*9-14*), biological (e.g., sexually transmitted infections [STIs]) (*15-17*), and non-behavioral (e.g., schistosomiasis, tuberculosis) (*18-20*) risk factors of HIV acquisition are also associated with antigenic stimulation. To integrate these observations, we posited the following immunologic framework: failure to adapt to varied repetitive or sustained antigenic stimuli, signifying unsuccessful immune allostasis, may erode immunologic integrity via a common immunologic program correlating with adverse outcomes in both HIV− and HIV+ persons (**Figure 1A**). We termed the program *age-independent immunosenescence* (AIIS) (**Figure 1A**). In the hypothesized immunologic framework, regardless of the underlying source or grade of antigenic stimulation, AIIS is an indicator of lower immunologic integrity.

Our hypothesis predicts that in HIV− persons, host antigenic burden gradually accumulates with age in response to repetitive, low-grade antigenic stimulation, ultimately inducing AIIS in a subset of those with AIIS susceptibility. Thus, age serves as a proxy, albeit imperfect, for cumulative host antigenic burden, rendering older age an AIIS risk factor (**Figure 1B**). In contrast, moderate-grade antigenic stimulation, for example, that associated with risk factors for HIV infection (e.g., risk behaviors), may precipitate a switch from AIIS-free to AIIS status at any age (**Figure 1B**). Hence, risk factor-induced AIIS may antedate and enhance the risk of HIV acquisition; post-HIV seroconversion, risk factor-induced AIIS may amplify immunologic damage associated with HIV infection,

increasing risk of AIDS as well as impaired treatment-associated immunologic reconstitution (**Figure 1A**).

In addition, our hypothesis predicts that, in HIV+ persons, high-grade antigenic stimulation associated with HIV viremia rapidly induces AIIS (**Figure 1B,C**). Unless immediately suppressed, sustained high-grade antigenic stimulation rapidly precipitates a switch from AIIS to advanced AIIS, which we posited should be more common in persons carrying variants in genes that correlate with increased HIV cell entry and/or replication (e.g., in the HIV coreceptor *CCR5* (*21-24*)). Thus, HIV+ persons may have AIIS attributable to three grades of antigenic stimulation (**Figure 1B**): low-grade, which occurs throughout aging; moderate-grade, which links to HIV risk factors; and high-grade, which is associated with HIV viremia. Hence, there may be two distinct barriers to restoration of AIIS-free status, independent of either the interval between HIV acquisition and ART initiation or the CD4+ count at ART initiation: (i) profound immunosuppression associated with advanced AIIS irreversibly damages the immune system, and (ii) AIIS associated with pre-existing or ongoing behavioral or non-behavioral risk factors for HIV infection is unresponsive to ART (**Figure 1B,C**).

A hallmark of the immune response to varied antigenic stimulation, especially viral infections, is expansion of CD8+ T-cells, accompanied or not by a decline in CD4+ T-cells (**Figure 1D**) (*25-29*). Allostasis is defined as the process of maintaining homeostasis through the adaptive change of the organism's internal environment in response to actual or perceived environmental and psychological stressors (*30, 31*). Applying this concept

to immune health, we surmised that successful calibration of immune function and preservation of immunologic resilience in response to repetitive or sustained antigenic stimuli depends on maintaining equilibrium between peripheral blood absolute CD8+ and CD4+ T-cell counts (CD8-CD4 equilibrium), rather than levels of either T-cell subset alone (**Figure 1D**). In this model, restrained expansion of CD8+ T-cells, regardless of CD4+ T-cell levels, reflects CD8-CD4 equilibrium and is a sign of successful immune allostasis. In contrast, unrestrained expansion of CD8+ T-cells, again regardless of CD4+ T-cell levels, reflects CD8-CD4 disequilibrium and is a sign of unsuccessful immune allostasis (**Figure 1D**). We considered CD8-CD4 equilibrium to be distinct from the CD4:CD8 T-cell ratio. Since this ratio is a mathematical construct, persons with the same CD4:CD8 T-cell ratio could have divergent CD4+ and CD8+ counts and thus, CD8-CD4 equilibrium or disequilibrium.

The framework of successful vs. unsuccessful immune allostasis predicts that immunologic integrity differs by CD8-CD4 equilibrium vs. disequilibrium status rather than HIV serostatus *per se*. In this framework, low- and moderate-grade, repetitive or chronic antigenic stimulation induces progressive degradation in CD8-CD4 equilibrium reflected by a transition from first-order equilibrium to disequilibrium, whereas sustained high-grade antigenic stimulation (e.g., with HIV virema) induces a transition from first-order to second-order equilibrium/disequilibrium states (**Figure 1E**). These transitions, correlating with progressive deteriorations in immune allostasis, result in greater accumulation of host antigenic burden and correspondingly lower immunologic integrity along a spectrum (best to worst**; Figure 1E**): first-order CD8-CD4 equilibrium (i.e., AIIS-free status) > first-

order disequilibrium (i.e., AIIS) ≥ second-order equilibrium > second-order disequilibrium (i.e., advanced AIIS). In this model, with suppression of antigenic stimulation, it is easier to reconstitute first-order equilibrium from a state of second-order equilibrium than from second-order disequilibrium.

To test this framework of immunologic integrity and predicted outcomes, we developed two parallel scales to capture a range of CD8-CD4 equilibrium/disequilibrium states, from AIIS-free (best) to advanced AIIS (worst) immune health status (**Figure 1D-F**). To gauge first-order equilibrium and AIIS, we developed four immune health grades (IHGs); to gauge second-order equilibrium and advanced AIIS, we developed four immune damage grades (IDGs) (**Figure 1F**). We also developed genomic (gene expression) metrics that distinguish AIIS-free from AIIS status. We applied the IHGs and IDGs as well as genomic metrics to large HIV− cohorts (children to >90 years) experiencing low- and moderate-grade antigenic stimuli (e.g., aging cohorts, female sex workers, renal transplant recipients), as well as primary and early HIV infection cohorts both before and after ART (**Figure 1G**; **Figure S1**; **Tables S1,S2**). Through this approach, we determined (i) whether AIIS is an antigen-activated, immunosenescence-mediating program; (ii) whether laboratory (IHG and IDG) and genomic metrics of the AIIS program are sensitive biomarkers of immunologic integrity linked to accumulated host antigenic burden, regardless of age or HIV serostatus; and (iii) to what extent inter-individual differences in proclivity to develop and reverse CD8-CD4 disequilibrium underpin the risk continuum of HIV acquisition, severe immune depletion, and impaired treatment-associated immune reconstitution, despite immediate ART.

RESULTS

Laboratory metrics of immunologic integrity

IHGs and IDGs were derived by co-indexing the CD4:CD8 T-cell ratio and CD4+ count using distinct cutoffs selected *a priori* (ratio values of 1.0 vs. 0.5 and 800 vs. 500 CD4+ cells/mm^3 in IHG vs. IDGs; **Figure 1F**). The cutoffs for the IHGs (ratio ≥1.0 vs. <1.0 and CD4+ count ≥800 vs <800 cells/mm^3) were based on the following observations. An inverted CD4:CD8 T-cell ratio was used as a cutoff because, irrespective of age, it is a mathematical reflection of expansion of CD8+ T-cells to levels that are uncompensated for by a concomitant increase in CD4+ levels. An inverted ratio correlates with impaired immune health status, disease risks, and mortality (*32-40*). Most HIV+ persons have an inverted ratio (*40-42*).

The CD4+ cutoff level to derive IHGs was based on our finding that the lower bounds of the interquartile range (IQR) of CD4+ counts in 16,126 HIV− persons was close to 800 cells/mm^3 [median: 952, IQR: 840 – 1,036; Ref. (*2, 3*) and **Supplementary Methods** and **Table S3**]. Thus, IHG-I and IHG-II signified preservation of first-order equilibrium, as these grades reflected lower CD8+ levels, with higher and lower CD4+ counts (≥800 and <800 cells/mm^3), respectively (**Figure 1F**). In contrast, IHG-III and IHG-IV signified first-order disequilibrium or AIIS, as these grades reflected higher CD8+ levels, with higher and lower CD4+ counts, respectively (**Figure 1F**).

For IDGs, the cutoffs were a ratio ≥0.5 vs. <0.5 and CD4+ count ≥500 vs. <500 cells/mm^3 (**Figure 1F**). The combination of a ratio of 0.5 and CD4+ count of 500 cells/mm^3 approximates the median levels at presentation (study entry/baseline) of 5,667 HIV+ adults from three separate primary/early HIV infection cohorts evaluated herein [median (IQR) ratio, 0.57 (0.38-0.82); median (IQR) CD4+ count, 500 cells/mm^3 (360-661); **Figure 1F**; **Table S1**). Additionally, 500 cells/mm^3 has been used as a cutoff level in clinical trials that evaluated the timing of ART in early asymptomatic HIV infection (*43, 44*). Thus, IDG-I and IDG-II signified preservation of second-order equilibrium, as these grades reflected lower CD8+ levels, with higher and lower CD4+ counts (≥500 and <500 cells/mm^3), respectively (**Figure 1F**). In contrast, IDG-III and IDG-IV signified second-order disequilibrium or advanced AIIS, as they reflected higher CD8+ levels, with higher and lower CD4+ counts, respectively (**Figure 1F**).

Hence, IHGs and IDGs represent parallel scales, as they gauge the relative extent to which CD8+ expansion is restrained vs. unrestrained in persons with higher and lower CD4+ levels (**Figure 1F**). IHG-I and IDG-I track equilibrium states signifying CD8^{lower}-CD4^{higher}; IHG-II and IDG-II track equilibrium states signifying CD8^{lower}-CD4^{lower}; IHG-III and IDG-III track disequilibrium states signifying CD8^{higher}-CD4^{higher}; and IHG-IV and IDG-IV track disequilibrium states signifying CD8^{higher}-CD4^{lower} (**Figure 1F**).

IHGs: laboratory features

Figure 2A shows median CD8+ and CD4+ T-cell counts by IHG status in the HIV− cohort from University of California, San Diego (UCSD). Corresponding values from the large

community SardiNIA cohort (n=3,896) and HIV− female sex workers (FSWs; n=1,050) from Kenya are shown in **Table S4**. The lowest and highest median CD8+ levels were in the IHG-II and IHG-III grades, respectively, whereas the highest and lowest median CD4+ levels were in the IHG-I and IHG-IV grades, respectively. Yet in the HIV− UCSD cohort, the median ratio was identical in IHG-III and IHG-IV (0.89), although these grades track significantly different levels of CD8+ and CD4+ T-cell counts (**Figure 2A**).

Response to ART is frequently classified based on level of CD4+ count restored (*45, 46*). CD4+ levels were similarly high in IHG-I and IHG-III (median 1063 and 930 cells/mm^3, respectively). Recovery of CD4+ counts to levels present in IHG-I and IHG-III would therefore signify CD4+ normalization (≥800 cells/mm^3), and hence, immunologic response. However, individuals classified as IHG-III had CD8+ counts nearly double those in IHG-I (median 1054 and 556 cells/mm^3, respectively) (**Figure 2A**).

AIIS in HIV− cohorts with low-grade antigenic stimulation

Considering age as an imperfect proxy for antigenic exposures accrued over time, we first evaluated IHG distribution by age in the low-risk, community-based SardiNIA cohort (age 18-102 years) (**Figure 2B**, far left). In younger adults, IHG-I was the most prevalent grade followed by IHG-II. With age, prevalence of IHG-I declined and IHG-II, IHG-III, and IHG-IV increased (**Figure 2B**; **Figure S2**). Compared with younger individuals, advanced age (≥80 years) is marked by an overrepresentation of IHG-II and AIIS; 14% of persons in the advanced age stratum (≥80 years) vs. <5% of persons in the youngest age stratum (18-29 years) had AIIS (P<0.001; **Figure 2B**).

AIIS in HIV− cohorts with moderate-grade antigenic stimulation

We next evaluated whether AIIS was induced by antigenic stimulation associated with non-behavioral (schistosomiasis) as well as behavioral and biologic (STIs) risk factors for HIV acquisition. In the non-behavioral cohort, 86% of HIV− Kenyan children with the highest *Schistosoma haematobium* egg counts in urine had IHG-IV compared with <10% in children without schistosomiasis (**Figure 2B**, left; *P*<0.001).

For the behavioral cohort, we evaluated 1,050 FSWs who were HIV− at baseline (**Figure S1**). We restricted analysis to FSWs with two HIV− test results at least 3 months apart and available laboratory data to compute IHGs (n=449; median baseline age: 32 years; IQR: 27-37 years; **Figure S1**; **Table S2**). An incrementally higher baseline behavioral activity score (BAS) (tracking condom use and number of clients) represented a proxy for higher risk factor-associated antigenic stimulation as well as HIV exposure and correlated with a step-wise increase in AIIS prevalence and reciprocally lower IHG-I prevalence at baseline (**Figure 2B**, middle). Baseline AIIS rates were (i) nearly 2.5 times higher in FSWs with higher (>0) compared with lower (<0) BAS scores (*P*<0.001), (ii) comparable in FSWs with lower BAS (11%) and older (14% in ≥80 years) HIV− persons without risk factors for HIV, and (iii) significantly higher in FSWs who during prospective follow-up subsequently seroconverted than in those who did not seroconvert (30% vs. 13%, *P*=0.009; **Figure 2B**).

AIIS with high-grade (HIV-associated) antigenic stimulation

HIV-associated antigenic stimulation was a potent inducer of AIIS, as most participants in the three HIV+ cohorts evaluated had AIIS at baseline, with most manifesting IHG-IV (**Figure 2B** right; **Figure S3**). In the primary HIV infection cohort from UCSD (PIC-UCSD; **Figure 1G**; **Figure S1**), 74% of participants recruited within 4 months of their estimated date of infection (EDI) were IHG-IV at presentation (**Figure 2B**, right). Additionally, among PIC-UCSD participants who were AIIS-free at study entry, the median time to develop AIIS was 2.89 months (95% confidence interval [CI], 2.56-4.37) (**Figure S3**).

HIV viral load (VL) served as a proxy for level of HIV-associated antigenic stimulation. In participants from the early HIV infection cohort (EIC; **Figure 1G**), progressively higher HIV VL at baseline correlated with incrementally higher rates of AIIS (**Figure 2B**, right; **Figure S4**). Among PIC-UCSD (**Figure S4**) and EIC (**Figure 2B**, right) participants presenting with lower VL (<1000 copies/mL), nearly 68% and 37%, respectively, presented with AIIS-free status. AIIS-free status was progressively higher in HIV+ participants from the EIC classified as spontaneous virologic controllers (SVC; also termed elite or viremic controllers (*47, 48*)) or long-term nonprogressors (LTNP) or both (**Figure 2B**, far-right). Nearly 50% of the participants with dual SVC and LTNP status preserved AIIS-free status at baseline.

AIIS resistance

Irrespective of grade of antigenic stimulation, some persons resisted progression to AIIS. AIIS-free status was observed in nearly 86% of older (≥80 years) individuals in the SardiNIA cohort, 14% of HIV− Kenyan children with higher *S. haematobium* eggs in urine

(≥500 eggs/mL), and 73% of HIV− FSWs with higher BAS (**Figure 2B**). FSWs who subsequently seroconverted classified into three groups (**Figure 2B**): Groups 1 and 2 were AIIS-free pre-infection; after HIV infection, Group 1 preserved this status, whereas Group 2 developed AIIS. In contrast, Group 3 had AIIS pre- and post-HIV infection. Thus, in the subset of FSWs for whom AIIS status was available pre and post infection, nearly 17% resisted AIIS attributable to both risk factors and HIV infection (Group 1), whereas 28% had AIIS pre- and post-infection (Group 3) (**Figure 2B**, middle).

AIIS-free status was preserved in 19% of PIC-UCSD participants recruited within 4 months of their EDI (**Figure 2B**) and 14% of EIC participants recruited within 12 months of their estimated date of seroconversion (EDS) (**Figure S3**). Although higher HIV VL correlated with higher AIIS rates, within each HIV VL stratum, a subset resisted HIV-induced AIIS (**Figure 2B**, right; **Figure S4**).

Induction and reversal of AIIS in HIV− persons

FSWs with higher behavioral and biological risk factors as indicated by a higher BAS or total STI score, respectively, were more likely to have AIIS at baseline (**Figure 2C**, purple interval plots). Among FSWs who remained HIV− during prospective follow-up, the hazard of switching from AIIS-free status at baseline to AIIS was significantly higher in those with a higher BAS at baseline; this was not the case for total STI scores (**Figure 2D**). This may relate to our finding that correlations between these two scores, while significant, are relatively low (r=0.26, P<0.001; **Figure S5**). Furthermore, the antigenic stimulation associated with behavioral activity is likely to be more frequent than that

associated with STIs (e.g., alloimmunization from repetitive exposure to client blood vs. episodic STIs).

In the 101 FSWs who remained HIV− for at least 4 years after study entry, AIIS was reversible (**Figure 2E**). During this interval, FSWs were encouraged to reduce risk behaviors, which correlated with significant reductions in BAS and total STI scores (**Figure S5**). Correspondingly, compared with baseline: (i) approximately 74% of those with IHG-III or IHG-IV reconstituted IHG-I or IHG-II, (ii) 57% of those with IHG-II reconstituted IHG-I, (iii) 16% with IHG-I developed IHG-II, and (iv) 6% developed AIIS (**Figure 2E**). Collectively, these findings suggest that, in HIV− FSWs, AIIS was an inducible and reversible trait, correlating with level of antigenic stimulation as proxied by behavioral activity and total STI scores.

First-order disequilibrium (AIIS) increases cancer and HIV risk

Younger HIV− FSWs and older HIV− individuals (≥80 years) shared a higher prevalence of AIIS (**Figure 2B**), suggesting that AIIS is an indicator of lower immunologic integrity. To determine the potential consequences of lower immunologic integrity in HIV− persons, we evaluated whether AIIS precedes and contributes to (i) cancer pathogenesis in persons without HIV risk factors and (ii) HIV acquisition in FSWs.

We determined the association between IHGs and cutaneous squamous cell cancer (CSCC) recurrence in long-term renal transplant recipients (RTRs). RTRs are at a heightened (up to 100-fold or more) risk of recurrent CSCC (*49*), providing an

experimental system to evaluate whether AIIS increases the risk of a second occurrence of CSCC. When we assessed our cohort of RTRs (*50*) (median age [IQR]: 66 [58-74] years; **Table S5**), 23% and 60% had IHG-I and IHG-II, respectively, and 18% had AIIS at initial evaluation (**Figure 2F)**. Thus, IHG-II was overrepresented in RTRs, likely reflecting an adaptive response to preserve CD8-CD4 equilibrium (i.e., suppression of CD8+ T-cell expansion in the face of declining CD4+ counts). Notably, a similar adaptive response was observed during aging (**Figure 2B**).

Forty long-term RTRs with a history of a single occurrence of CSCC were evaluated for development of a second CSCC occurrence during prospective follow-up (**Table S5**). RTRs with IHG-I at baseline resisted a second occurrence of CSCC, whereas those with IHG-II or AIIS had intermediate and the highest risks of a second occurrence, respectively (**Figure 2G**). Duration of immunosuppression or age did not differ by IHG status (**Table S5**). These findings suggest that (i) persons vary in their proclivity to develop AIIS in response to antigenic stimulation (alloimmune responses) and (ii) those preserving AIIS-free status, especially IHG-I, may have superior immunologic integrity, as reflected by resistance to develop CSCC.

We next evaluated the association of AIIS status at baseline with subsequent HIV seroconversion in FSWs, before and after controlling for behavioral and biologic risk factors. The odds of HIV seroconversion were significantly higher in FSWs with higher baseline BAS and total STI scores (**Figure 2C**, blue interval plots). Baseline IHG-III and IHG-IV also associated with future HIV seroconversion risk (**Figure 2C**, blue interval

plots). The association of IHG-IV at baseline with future HIV seroconversion [unadjusted odds ratio (OR), 3.76; 95% CI, 1.52-9.31] was stronger than with IHG-III (unadjusted OR, 2.28; 95% CI, 0.96-5.41) (**Figure 2C**; **Table S6**). AIIS at baseline was an independent determinant of future HIV seroconversion, as in a multivariate analysis (**Table S6**), after controlling for age as well as BAS and total STI scores, the association of IHG-IV with future HIV seroconversion persisted (adjusted OR, 2.97; 95% CI, 1.05-8.38). These findings suggest that FSWs with a proclivity to switch from AIIS-free status to IHG-III and, especially IHG-IV, in response to risk factor-associated antigenic stimulations, were at a significantly higher risk of acquiring HIV vs. those preserving AIIS-free status.

Ranking accumulated host antigenic burden

Figure 2B illustrates that IHG distributions capture the population-level association of different grades of antigenic stimulation with host antigenic burden accumulated. Based on AIIS prevalence (**Figure 2B,F**), we ranked host cumulative antigenic burden in subsets of HIV− and HIV+ populations (**Figure 2H**). In this ranking system, the two extremes of cumulative host antigenic burden in HIV− populations were: younger, low-risk HIV− persons and children with severe schistosomiasis (**Figure 2H**). We used this system to rank residual host antigenic burden (AIIS) in HIV+ persons during ART and identify factors that shift the rank leftward toward younger HIV− persons.

Second-order CD8-CD4 disequilibrium in therapy-naïve HIV+ persons

In our model (**Figure 1E,3A**), soon after HIV infection, most persons with or without AIIS-free status before infection manifest AIIS (IHG-III or IHG-IV) and concomitantly, segregate

into those with second-order equilibrium (IDG-I or IDG-II) vs. disequilibrium, i.e., advanced AIIS (IDG-III or IDG-IV). In both HIV+ persons enrolled within 4 months of their EDI (PIC-UCSD cohort) or 12 months of their EDS (EIC cohort), approximately 60% vs. 40% of participants had IDG-I or IDG-II vs. IDG-III or IDG-IV, respectively, at baseline (**Figure 3B**; **Figure S3**). By evaluating contemporaneous IHGs and IDGs in EIC participants (**Figure 3C**, left), we defined the immunologic disadvantage of having IHG-IV at baseline (study entry). At baseline, approximately 86% of EIC participants (n=4,883) had AIIS; of these, 90% had IHG-IV (n=3,811; **Figure 3C**, left). Of the EIC participants with IHG-IV at baseline, nearly 28% had IDG-I and 39% had IDG-IV (**Figure 3C**, left). In contrast, among EIC participants with IHG-I, IHG-II, and IHG-III at baseline, 100%, 76%, and 90%, respectively, had IDG-I (**Figure 3C**, left).

Based on these findings (**Figure 3C**, left), we next determined whether preservation of first-order equilibrium (IHG-I or IHG-II) at baseline associated with preservation of second-order equilibrium (IDG-I or IDG-II) during therapy-naïve HIV disease course. To test this proposition, we focused on the 975 EIC participants in whom four consecutive years of therapy-naïve laboratory data were available (**Figure 3C**, right). Most HIV+ persons with IHG-I or IHG-II at baseline preserved IDG-I or IDG-II during a 4-year therapy-naïve follow-up period (**Figure 3C**, right). In contrast, persons with IHG-III or IHG-IV at baseline were more likely to progress to advanced AIIS. Thus, compared to persons with baseline IHG-I, the likelihood of having advanced AIIS in year 4 of therapy-naïve disease course was higher by 3.48- and 10.98-fold in individuals with baseline IHG-III and IHG-IV, respectively (**Figure 3D**). Hence, while IHG-I and IHG-III are characterized by higher

CD4+ counts (**Figure 1F,2A**; **Table S4**), the proportion of individuals in these sets of IHGs who progressed to advanced AIIS differed substantially (**Figure 3C**, right). Collectively, these findings suggest that persons preserving first-order equilibrium after HIV infection have an immunologic advantage, as they resisted progression to advanced AIIS during the therapy-naïve disease course.

Second-order CD8-CD4 disequilibrium in HIV− FSWs

Nearly 9% of HIV− FSWs with AIIS at baseline manifested IDG-III or IDG-IV (**Figure S6**), suggesting that even in the absence of HIV infection, increased antigenic stimulation associated with HIV risk factors predisposes to development of advanced AIIS. We found that the odds of presenting with IDG-I after HIV infection were 73% lower in FSWs who had IHG-IV compared to IHG-I before infection (OR, 0.27; 95% CI, 0.05-1.38). Together, these and other findings in FSWs who seroconverted (**Figure 2B**,**C**) as well as in HIV+ patients accrued during primary/early HIV infection (**Figure 3C,D**) provide evidence in support of a continuum: at the time of viral exposure, disequilibrium in at-risk individuals increases the risk of HIV acquisition; if infection were to occur, pre-infection CD8-CD4 equilibrium/disequilibrium status contributes to post-infection equilibrium/disequilibrium status.

CD8-CD4 equilibrium vs. CD4+ status as predictors of AIDS

Additional lines of evidence support that preservation of first- or second-order equilibrium confers an immunologic advantage in HIV+ persons. First, the hazard of developing AIDS (1993 CDC criteria (*51*)) in EIC participants reflected their IHG or IDG grades at

enrollment: IV > III ~ II > I (**Figure 3E**; **Figure S7** depicts results by 1987 CDC criteria of AIDS (*52*)). Second, the proportion of EIC participants with anergy (assayed by delayed-type hypersensitivity skin tests) at baseline was greater in those with IHG-III or IHG-IV at enrollment (**Figure S8**). Third, HIV VL in EIC or PIC-UCSD participants was lower in those preserving first- or second-order equilibrium, and lowest in those with IHG-I or IDG-I (**Figure 3E**; **Figure S4**).

In contrast, the CD4+ count and the CD4:CD8 T-cell ratio were inferior indicators of immune status. First, grades with higher (IHG or IDG I and III) or lower (IHG or IDG II and IV) CD4+ counts (**Table S4,S7**) were associated with divergent AIDS risks (**Figure 3E**). Second, VL was lowest in IHG or IDG I and highest in IHG or IDG IV, whereas CD4+ counts within the IHGs or IDGs lacked a hierarchical relationship with VL (**Figure 3E**). Third, while progressively lower CD4+ counts at baseline correlated with an incrementally higher risk of developing AIDS (1993 CDC criteria (*51*)), within each CD4+ stratum, those preserving second-order equilibrium (IDG-I or IDG-II) had a lower risk than those with IDG-III or IDG-IV (**Figure 3F**; **Figure S9** depicts results by 1987 CDC criteria of AIDS (*52*)). Finally, each ratio stratum (e.g., <0.25, 0.25 to <0.50, 0.50 to <0.75 and ≥0.75) conflates individuals with two IDGs that associate with contrasting risks of developing AIDS (**Figure S9**).

IDGs track distinct HIV disease endotypes

Progressive lowering in immunologic integrity in therapy-naïve HIV+ patients is traditionally thought to relate to a progressive decline in CD4+ T-cells. However, the

abovementioned data suggest that the CD4+ count is an imprecise biomarker of immune status and raised the prospect that CD8-CD4 second-order equilibrium/disequilibrium status at baseline and changes (IDG switches) during therapy-naïve disease course may be more precise indicators of immunologic integrity (model in **Figure 4A**, top). We tested this hypothesis in EIC participants who were therapy-naïve for at least 3 years and met other criteria noted in the Figure 4B schema (n=2,106). EIC participants with IDG-I vs. IDG-II or IDG-III at baseline were least likely to progress to IDG-IV (**Figure S10**). A switch from second-order equilibrium (IDG-I or IDG-II) at baseline to advanced AIIS (IDG-III or IDG-IV) during three years of follow-up without ART associated with faster progression to AIDS by either the 1993 or 1987 criteria (**Figure 4A**; **Figure S10**). A spontaneous switch from second-order disequilibrium to second-order equilibrium was associated with improved immunologic integrity, as indicated by slower rates of progression to AIDS (**Figure 4A**, far right). Similar associations were observed among persons stratified according to strata of baseline CD4+ counts (**Figure S10**).

These data suggest that a switch from a better to a worse IDG grade, signifying failure to preserve CD8-CD4 equilibrium, marks a deterioration in immunologic integrity (increased AIDS risk). Preservation of equilibrium requires two concurrent events: restrained CD8+ T-cell expansion and proportional changes in CD4+ levels. To test this proposition, we evaluated CD4+ and CD8+ T-cell count trajectories in ART-naïve persons presenting with IDG-I or IDG-II who either preserved those grades or switched to a better (IDG-II→IDG-I) or worse (IDG-I or -II → IDG-III or IV) IDG grade. Reflecting both events, the CD8+ and CD4+ trajectories (red and blue) were closely approximated in EIC and PIC participants

preserving IDG-I or IDG-II during ART-naïve disease course (**Figure 4B**; **Figure S11**). In contrast, a switch from IDG-I or IDG-II to a worse IDG grade was associated with CD8+ and CD4+ trajectories that were more widely separated (**Figure 4B**; **Figure S11**).

IDG switches tracked HIV VL, as VL was higher in persons who switched from second-order equilibrium to second-order disequilibrium and lowest in those preserving or spontaneously restoring IDG-I (**Figure 4B,** green plots; **Figure S11**; **Table S8**). Hence, CD8-CD4 equilibrium/disequilibrium states defined by IDGs I to IV classify four distinct HIV disease endotypes correlating with VL, CD4+ and CD8+ trajectories, and clinical outcomes during the therapy-naïve disease course (**Figure 4B**, bottom schema). Presentation with and preservation of IDG-I during the therapy-naïve disease course associated with the best outcomes: (i) lowest AIDS risks and VL (**Figure 3E, 4A-B**) and (ii) greater preservation of first-order equilibrium (IHG-I or IHG-II) at baseline and during the disease course (**Figure S12**). Collectively, these findings suggest that IDGs I to IV track HIV endotypes, grouping individuals with progressively greater accumulation of host antigenic burden, worsening immunologic integrity, and increasing VL (**Figure 4B**, bottom schema).

Immunologic and virologic attributes of AIIS-free status

First-order CD8-CD4 equilibrium, i.e., AIIS-free status (IHG-I or IHG-II), is the most common equilibrium status in HIV− adults (**Figure 2B**). While IHG-I and IHG-II track CD4+ counts above and below 800 cells/mm^3, respectively (**Figure 1F,2A**), the IHG distributions in HIV− populations suggest that reconstitution of AIIS-free status rather than

normalization of CD4+ counts (>800 cells/mm^3) is a more optimal immunologic status to achieve during ART. To test this viewpoint, we determined the association of IHGs with immunologic and virologic attributes in EIC and/or PIC participants (**Tables S9-S11**). Expression of phosphorylated STAT5 in T-cells after IL-7 stimulation was considered an attribute of T-cell responsiveness, as an intact IL-7/IL-7R (CD127) axis is essential for immunologic integrity (*53, 54*). Proportions of CD4+ T-cells expressing programmed cell death (PD)-1 and HLA-DR+ CD4+ effector memory T-cells were considered attributes of T-cell dysfunction and activation, respectively (*54-57*). These three attributes and proportion of T-cells expressing the IL-7 receptor (CD127) were evaluated in eight groups: Group 1, HIV− persons with IHG-I or IHG-II; Groups 2-6, HIV+ persons categorized according to IHG reconstituted during VL-suppressive ART and serostatus for cytomegalovirus (CMV); and Groups 7-8, HIV+ persons with higher HIV VL due to ineffective or no ART.

In HIV+ persons with ART-induced VL suppression, reconstitution of AIIS-free status (Groups 2-4) vs. failure to restore this status (Groups 5-6) associated with levels of T-cell responsiveness, activation, and dysfunction, and proportions of T-cells bearing the IL-7 receptor closer to those in Group 1 (**Figure 5A**; **Figure S13**). Thus, levels of these immunologic attributes were similar in AIIS-free subjects, irrespective of HIV or CMV serostatus. In contrast, comparing HIV+ persons who did or did not have CD4+ normalization (IHG-I and IHG-III [groups 3 and 5] vs. IHG-II and IHG-IV [groups 4 and 6]) conflated HIV+ persons who did vs. did not achieve AIIS-free status, obscuring true immunologic reconstitution (**Figure 5B**; **Figure S13**). Restoration of AIIS-free status in

HIV+ persons also associated with other salutary attributes: (i) lower plasma IL-6 levels (**Figure 5C**), (ii) greater responsiveness to hepatitis B virus vaccine administered during ART (**Figure 5D**), and (iii) lower HIV DNA levels (**Figure S13**) in peripheral blood mononuclear cells of individuals on virally suppressive ART.

Genomic metrics of AIIS status

Our model (**Figure 1A,B**) predicts that varied antigenic stimuli activate the AIIS program mediating a lowering in immunologic integrity. To test this proposition, we determined whether stimuli experienced in association with age (e.g., viral infections other than HIV) and HIV infection induce the expression of a transcriptomic (gene expression) profile that tracks well-documented immunosenescence-associated attributes and AIIS status. We derived two gene signatures: AIIS-1 and AIIS-2. AIIS-1 comprised 215 genes that correlated with 4 markers of immunosenescence: CD4+ and CD8+ senescent T-cells (CD28− CD4+ or CD8+ T-cells), T-cell receptor excision circles, and telomerase reverse transcriptase (*58*) (**Table S12**). AIIS-2 comprised 110 genes that differentiated AIIS-free from AIIS status at high stringency (area under the curve, ≥0.80; **Table S12**; Supplementary methods). We computed the gene signature scores, reflecting the relative expression levels of genes within AIIS-1 and AIIS-2, in 8 study groups (**Figure 5E,F**) and in 15 publicly available gene expression datasets relevant to understanding the extent to which the AIIS program is transcriptionally activated in settings of acute (e.g., viral infections) and repetitive (e.g., with age) low-grade and high-grade (e.g., HIV infection) antigenic stimulation and conditions that, in HIV− persons, are associated with lower immunologic integrity (e.g., tuberculosis) (**Figure 5G-O**; **Figure S14**). The study design,

sample sources, and microarray platforms used in these datasets are presented in **Table S13**.

Gene signature scores of AIIS-1 and AIIS-2 showed similar patterns and were lower in study groups with AIIS-free status (irrespective of HIV or CMV serostatus), compared with the 4 groups with AIIS who comprised individuals receiving virally suppressive ART that failed to reconstitute AIIS-free status and therapy-naïve persons with IHG-IV or those classified as spontaneous virologic controllers (compare last 4 vs. first 4 groups; **Figure 5E,F**). Since both scores distinguished AIIS from AIIS-free groups, henceforth, we show results with the AIIS-2 score (AIIS-1 score results are presented in **Figure S14**).

Evaluation of scores pre- and post-ART indicated that suppression of HIV-associated antigenic stimulation lowered scores (**Figure 5G**). However, scores were similar in those who did vs. did not achieve CD4+ levels ≥500 cells/mm^3 (**Figure 5G**). Scores were higher in CD4+ T-cells isolated from HIV+ persons on ART that failed to suppress VL (VL > 50 copies/mL) (**Figure 5H**). These results suggest that suppression of HIV-associated antigenic stimulation with ART induced a lowering in AIIS-1 and AIIS-2 scores.

In three separate HIV− cohorts, AIIS-1 and AIIS-2 gene signature scores increased progressively with age (**Figure 5I-J**; **Figure S14**). This increase may relate to the cumulative effect of repetitive transcriptional activation of the AIIS program, as (i) transient antigenic stimuli associated with viral infections (e.g., Epstein-Barr virus, influenza infection) in adults induced transient increases in the gene signature scores (**Figure 5K,L**,

Figure S14), and (ii) scores were higher in children with acute respiratory syncytial virus infection, correlating with infection severity (**Figure 5M**).

Compared with controls, gene signature scores were higher in persons with idiopathic CD4+ lymphocytopenia, an immunosuppressive condition associated with diseases similar to those in HIV+ persons (*59*) and intermediate in HIV− individuals with sarcoidosis (**Figure 5N**; **Figure S14**). Scores overlapped in older HIV− persons and younger adults with conditions correlating with increased accumulation of host antigenic burden (**Figure 5I**): HIV+ individuals, irrespective of ART status and HIV− persons with latent tuberculosis infection and active pulmonary tuberculosis. We mitigated methodologic confounding by ensuring that cellular sources (whole blood) and microarrays used for assessment were identical and by concurrently normalizing expression datasets.

HIV viremia was a potent inducer of AIIS, as reflected by comparison of pre- and post-infection IHGs in FSWs (**Figure 2B**). To corroborate this finding, we evaluated asymptomatic HIV+ persons with undetectable HIV VL who were receiving ART and volunteered for analytical treatment interruption after receipt of four doses of a dendritic cell-based vaccine (*60*). HIV viremia was detectable after ART interruption and was associated with an increase in the expression of genes that correlated with IFN expression as well as senescent CD28− CD4+ or CD8+ T-cells (components of AIIS-1 score) and the AIIS-2 gene signature score (**Figure 5O**; **Figure S14**; **Tables S12-S14**).

Collectively, these data (**Figure 5**) suggest that (i) antigenic stimulation associated with HIV infection and non-HIV sources induces transcriptional activation of the AIIS program, (ii) immunologic and transcriptional profiles were more closely aligned to CD8-CD4 equilibrium status rather than HIV or CMV serostatus or age, and (iii) the optimal immunologic benchmark to achieve during ART is reconstitution of AIIS-free status, especially since this is also the most common status in younger, HIV− adults (**Figure 2B**).

Model of immune reconstitution tested

These data (**Figure 5**), including those showing the confounding that may occur when using CD4+ counts as a biomarker of level of immune reconstitution, provided the impetus to test a new immune reconstitution model. In this model, immune reconstitution in HIV+ persons is conditional on and occurs along parallel equilibrium states vs. an increase in CD4+ counts. First (IHG-I or IHG-II)- and second- (IDG-I or IDG-II) order equilibrium are parallel equilibrium states, whereas AIIS (IHG-III and IHG-IV) and advanced AIIS (IDG-III and IDG-IV) are parallel first- and second-order disequilibrium states (**Figure 1F**). We therefore posited that the chances of rapidly reconstituting first-order equilibrium (AIIS-free status) is more likely in persons initiating ART with second-order equilibrium than advanced AIIS. Per this posit, initiation of ART soon after HIV infection at higher CD4+ counts is insufficient in overcoming the constraints that advanced AIIS has on reconstitution of AIIS-free status. In turn, failure to rapidly reconstitute AIIS-free status predisposes to development of AIDS and non-AIDS comorbidities (e.g., cancer) during ART. We evaluated this immune reconstitution model in participants from three HIV cohorts with distinct epidemiologic characteristics, but in whom we could account for the

interval between HIV acquisition and initiation of ART. In this manner, we mitigated the confounding that occurs when using higher CD4+ counts as a proxy for early HIV disease stage (*43, 44*).

Reconstitution of immune health by pre-ART IDG

In the three cohorts, achievement of AIIS-free status was more closely related to the IDG at pre-ART rather than CD4+ count at ART initiation or timing of ART (**Figure 6A**). Although the median interval between acquisition of HIV and initiation of ART was progressively longer across the 3 cohorts [PIC-UCSD < EIC < and PIC cohort from CAPRISA (PIC-CAPRISA)], the hierarchy of the likelihood and rate of achieving AIIS-free status by pre-ART IDG was consistent across cohorts: IDG-I ≥ IDG-II > IDG-III > IDG-IV (**Figure 6A**; **Figures S15-17**). A similar hierarchy was observed in PIC-UCSD and EIC participants initiating ART earlier or later (**Figures S15,S16**). Earlier ART in the PIC-UCSD was initiation of ART within 4 months of EDI; in the EIC, it was initiation of ART within 12 months of EDS (*2, 3*).

The hazard ratio for developing serious clinical events (e.g. cancer) during ART was approximately 8- to 9-fold higher in persons starting ART at IDG-III or IDG-IV compared with IDG-I (**Figure 6B**; **Table S15**). The START clinical trial (*44*) indicated that initiation of ART at higher CD4+ counts mitigated development of serious clinical events during ART. However, we observed that, despite similarly high CD4+ counts (≥500 cells/mm^3) at pre-ART (IDG-I vs. IDG-III), patients with IDG-III at pre-ART had a nearly 9-fold higher hazard of developing serious clinical events than IDG-I (**Figure 6B**) and a constrained

capacity to reconstitute AIIS-free status (**Figure 6A**). To resolve this discrepancy, we grouped EIC participants in a manner that would mirror the groups generated by the randomization strategy used in the START clinical trial (*44*). The START trial randomized participants presenting with CD4+ counts ≥500 cells/mm^3; ART was initiated either immediately or deferred until CD4+ counts declined to <350 cells/mm^3. Similarly, we categorized EIC participants with baseline CD4+ ≥500 cells/mm^3 according to starting ART (i) at ≥500 CD4+ cells/mm^3 (comparable to the immediate-initiation arm of START), (ii) when counts had declined to <350 CD4+ cells/mm^3 (comparable to the deferred-initiation arm of START), or (iii) when counts had declined to levels between 350 and 500 CD4+ cells/mm^3 (what we designated as the intermediate initiation arm).

Predictably, the interval between EDS and ART initiation was progressively shorter in the deferred-, intermediate-, and immediate-initiation arms (**Figure 6C**). Although these arms were associated with incrementally faster rates of reconstituting AIIS-free status (**Figure 6C**, left), each conflated individuals with distinct IDGs. In each arm, rates and chances of reconstituting AIIS-free status were faster and greater when initiating ART at IDG-I or IDG-II (**Figure 6C,D**). Thus, differences in the odds of reconstituting AIIS-free status occurred despite the fact that the CD4+ counts at ART initiation in the immediate-initiation arm were similarly high (≥500 CD4+ cells/mm^3) in persons with IDG-I or IDG-III (634 or 620 cells/mm^3) and in the deferred-initiation arm were similarly low (<500 CD4+ cells/mm^3) in persons with IDG-II or IDG-IV (301 or 288 cells/mm^3) (**Figure 6D**).

Immune reconstitution endotypes

We used the rankings of accumulated host antigenic burden in HIV− study populations (**Figure 2H**) as a guidepost for defining the level of residual host antigenic burden in HIV+ populations receiving virally suppressive ART. We posited that the HIV disease endotypes defined by the IDGs (**Figure 4B**) also represent immunologic reconstitution endotypes. In this model, regardless of the interval between HIV acquisition and ART initiation, the IDG present at time of initiation of ART predicts level of residual host antigenic burden, i.e., proportion of individuals in the study population with residual AIIS (IHG-III or IHG-IV).

While lengthening durations of ART associated with step-wise increases in the proportion of individuals reconstituting IHG-I or IHG-II, the increase was more rapid in the two groups that initiated ART closer to the time of HIV acquisition (PIC-UCSD and earlier ART in EIC) (**Figure 7A**). However, in the latter two groups who initiated ART proximal to HIV acquisition (**Figure 7B**) or those starting ART later (**Figure S18**), the relative proportions of the IHGs that were reconstituted during ART were conditional on the IDG at pre-ART.

Initiation of ART with IDG-I and IDG-II supported reconstitution mainly of IHG-I and a mixture of IHG-I and IHG-II, respectively. Pre-ART IDG-III supported reconstitution of IHG-III and IHG-I during the early and later years of ART, respectively (**Figure 7B**; **Figure S18**). Thus, irrespective of the interval between HIV acquisition and initiation of ART, the proportions of the IHGs reconstituted in PIC-UCSD and EIC participants who had received at least 4 years of ART and initiated ART in the presence of IDG-I and IDG-II closely mirrored that of low-risk younger (18-29 years) and older (≥80 years) HIV− adults,

respectively (**Figure 7B**; **Figure S18**). In contrast, initiation of ART in the presence of IDG-IV associated with muted reconstitution of AIIS-free status, despite early ART initiation (**Figure 7B**). For example, only 40% of EIC participants who initiated ART early (<12 months from EDS) in the presence of IDG-IV had reconstituted IHG-I or IHG-II after 4 years of ART; the remainder had nearly equal proportions of IHG-III or IHG-IV (**Figure 7B**, top far right). The latter IHG distribution pattern mirrored that of HIV− FSWs with a higher BAS (**Figure 7B**).

Differences in IHG reconstitution patterns by IDG status at pre-ART could not be attributed to wide differences in age (**Figure 7B**). Thus, IDGs correlating with the lowest (IDG-I), moderate (IDG-II), and highest (IDG-IV) host antigenic burden accumulated before initiation of ART (**Figure 4B**) reconstituted the IHG distribution patterns of three groups of HIV− persons who have the lowest, moderate, and higher accumulated host antigenic burden, respectively (**Figure 2H,7C**).

Intrinsic immunologic advantage and acquired disadvantage

Advanced AIIS (IDG-III or IDG-IV) was an immunologic disadvantage, as initiation of ART in the presence of these grades thwarted rapid reconstitution of AIIS-free status (**Figure 6A,C,D** and **Figure 7B**) and associated with an increased hazard of serious adverse events during ART (**Figure 6B**). We posited that this disadvantage has 3 potential attributions: (i) pre-existing advanced AIIS (attributable to risk factors for HIV as observed in HIV− FSWs; **Figure S6**); (ii) intrinsic immunologic disadvantage (attributable to carriage of detrimental host genetic variants that enhance HIV entry/replication, and the

corresponding increase in viremia-associated antigenic stimulation predisposes individuals to rapidly progress to advanced AIIS); and (iii) acquired immunologic disadvantage (attributable to delayed ART, as sustained high-grade HIV-associated antigenic stimulation drives a switch from IDG-I or IDG-II to advanced AIIS). Hence, we hypothesized that reversing the negative effects that pre-existing immunologic disadvantage may have on reconstitution of AIIS-free status requires lowering of risk factor-associated antigenic stimulation (e.g., **Figure 2E**); reversing the negative effects of acquired immunologic disadvantage requires starting ART before a switch to advanced AIIS occurs; however, the negative effects of an intrinsic disadvantage may be irreversible despite earlier ART. We could not evaluate the impact of pre-HIV immunologic disadvantage on reconstitution of AIIS-free status, but were able to evaluate the impacts of an intrinsic and acquired immunologic disadvantage. To accomplish this, we determined the rate and odds of reconstituting AIIS-free status in PIC-UCSD and EIC participants stratified into 8 groups (**Figure 8A,B**) and performed genetic studies (**Figure 8C,D**).

These 8 groups differed according to their IDG at entry (baseline) and pre ART, as well as timing of ART. Groups 1-4 vs. 7-8 represent individuals with an immunologic advantage vs. disadvantage at entry (**Figure 8A**). Groups 7 and 8 presented with advanced AIIS and initiated ART with that status, and they manifested diminished rates and odds of reconstituting AIIS-free status regardless of ART timing (**Figure 8A,B**; **Figure S19**). In EIC participants with the same IDG at baseline and starting ART within 12 months of EDS, those with IDG-III and IDG-IV at baseline and pre-ART had no significant changes

in laboratory values at these two timepoints and exhibited an 85% and 70% lower incidence rate ratio of achieving AIIS-free status, respectively, compared with those with IDG-I at baseline and pre-ART (**Figure S20**).

Groups 5 and 6 represent rare individuals who acquired an immunologic advantage by spontaneously switching from advanced AIIS to second-order equilibrium (IDG-I or IDG-II) before starting ART (**Figure 8A,B**). Group 4 acquired an immunologic disadvantage, as they initiated ART later after a switch to IDG-III or IDG-IV had occurred; this associated with muted AIIS-free reconstitution (**Figure 8A,B**; **Figure S19**). While Group 3 also acquired an immunologic disadvantage, they initiated ART early; this associated with preserved capacity to rapidly reconstitute AIIS-free status. Groups 1 and 2 initiated ART early and late, respectively, but with IDG-I or IDG-II; this associated with rapid AIIS-free reconstitution. In persons with an immunologic advantage at entry, each additional year of untreated infection was associated with a step-wise reduction in the likelihood of reconstituting AIIS-free status (**Figure S21**). Most serious adverse events occurred in the groups with an intrinsic (Groups 7 and 8) or acquired (Group 4) immunologic disadvantage (**Figure 8B**). Thus, both the intrinsic and acquired immunologic disadvantage as well as ART timing influenced the odds and pace of optimal immunologic reconstitution defined as achievement of AIIS-free status.

Genetic determinants of advanced AIIS

To assess the genetic contributions to intrinsic disadvantage, we used a genetic score (distinct from the gene signature score in Figure 5E-O) to quantitate the number of alleles

(polymorphisms) well documented as beneficial or detrimental based on their associations with HIV cell entry/replication (*21-24, 61-63*) and AIDS development; these alleles are in the HIV coreceptor gene *CCR5* and major histocompatibility locus (e.g., *MICA* gene; **Table S16**). The IDGs were not equally distributed by genetic score; instead, a gradient effect was observed (**Figure 8C**). Thus, a lower genetic score signifying a disproportionately lower number of beneficial than detrimental alleles associated with a predisposition to present with IDG-IV and a higher HIV VL (**Figure 8C**; **Figure S22**).

At baseline, IDG-I was disproportionately overrepresented in persons presenting with IHG-I, whereas IDG-IV was disproportionately overrepresented in persons presenting with IHG-IV (**Figure 3C**). Correspondingly, these extremes associated with the maximal vs. least restriction of HIV replication and hazard of progressing to AIDS (**Figure S23**). Paralleling this, the proportion of beneficial vs. detrimental alleles was disproportionately overrepresented in persons who preserved IHG-I and IDG-I at baseline and underrepresented in persons with IHG-IV and IDG-IV; these groups also associated with the lowest and highest VL (**Figure 8D**, left; **Figure S23**).

Switching from an immunologic advantage to disadvantage and vice versa also had a genetic basis. Two groups manifested a higher genetic score (more beneficial than detrimental alleles): (i) persons who resisted a detrimental switch from IDG-I to any other IDG grade and (ii) those with a beneficial switch, i.e., individuals who spontaneously switched from IDG-IV at baseline to a more beneficial IDG grade (**Figure 8D**, right).

Discussion

There are three key findings of our study. First, in response to varied sources of repetitive or chronic antigenic stimulation, cycles of immune injury and repair ensue, driving bidirectional shifts between CD8-CD4 equilibrium and disequilibrium states. Macro level equilibrium:disequilibrium shifts can be quantified and monitored through laboratory metrics (IHGs and IDGs) that track the extent of CD8+ T-cell expansion, relative to CD4+ T-cell levels; micro level equilibrium:disequilibrium shifts are quantifiable via genomic metrics (AIIS-1 and AIIS-2 gene signatures) that track well-established immunosenescence markers or differentiate AIIS from AIIS-free status.

Second, incomplete immune repair, despite clearance or suppression of the source of immune injury, results in accumulation of host antigenic burden and eventually unsuccessful immune allostasis expressed as first-order (AIIS) or second-order (advanced AIIS) disequilibrium. Consequently, conditions that correlate with repetitive or chronic antigenic stimulation share two hallmarks: (i) macro- and micro-level metrics reflective of CD8:CD4 disequilibrium, and (ii) lower immunologic integrity. We show that use of conventional laboratory metrics of immune status (CD4+ count) or age to infer/assess immunologic integrity may have resulted in confounding in both clinical practice and intervention trials.

Third, relevant to understanding HIV/AIDS pathogenesis and treatment-associated outcomes, disequilibrium states of AIIS and advanced AIIS can be pre-existing in persons with behavioral, biological (STIs), and non-behavioral (e.g., schistosomiasis) risk factors

for HIV or emerge rapidly after HIV infection. Disequilibrium states negatively contribute to the risk/severity continuum, as they (i) heighten risk of HIV acquisition, (ii) increase risk of developing AIDS in therapy-naïve persons and serious adverse events during ART, and (iii) constrain the pace and capacity to reconstitute AIIS-free status, even if ART is initiated soon after HIV acquisition at higher CD4+ counts. Thus, pre-infection CD8-CD4 equilibrium status may substantially contribute to post-infection outcomes, pre- and post-ART. Some persons resist developing AIIS or advanced AIIS, mediating inter-individual differences in outcomes along this continuum.

This new framework of immunologic heath has broad implications for understanding the basis and outcomes of non-HIV diseases. First, successful immune allostasis reflects adaptation to inflammation and immune responses associated with varied antigenic stimuli, including those commonly experienced throughout life. Regardless of the severity of antigenic stimulation, successful immune allostasis signifies preservation of the equilibrium between CD8+ and CD4+ T-cells rather than the absolute values of these measures. Second, degradation in CD8-CD4 equilibrium leading to AIIS can occur quickly or over a lifespan as a consequence of repetitive, routine, low-grade antigenic challenges, suggesting that age is an imperfect proxy for accumulated host antigenic burden. Reflecting this, the degree of transcriptional activation of the AIIS program monitored by genomic metrics was lower in AIIS-free persons, irrespective of their HIV serostatus, compared to individuals with AIIS; however, genomic metric levels were similar in older HIV– and younger adults with conditions that associate with increased accumulation of host antigenic burden and inflammation (HIV, tuberculosis). The latter finding may

undergird the observation that older HIV– and younger HIV+ adults on long-term ART share risk for similar outcomes (e.g., cancer, cardiovascular diseases (*4, 5*)). Extreme cases of chronic antigenic stimulation leading to advanced AIIS in the absence of HIV might explain idiopathic CD4+ T-cell lymphocytopenia.

Heretofore, CD4+ depletion has been viewed as the *sine qua non* correlate of lower immunologic integrity in HIV infection. Correspondingly, since the start of the epidemic, CD4+ counts have been used to stage immune status; design studies of pathogenesis, genome-wide associations, and clinical trials; predict outcomes; and define AIDS (*43-46, 51, 62, 64, 65*). Recent studies have suggested use of the CD4:CD8 T-cell ratio as a metric of immune status (*40-42*). However, our data suggest that use of these measures can lead to confounding, as persons with the same CD4+ count or ratio may have contrasting CD8-CD4 equilibrium states, correlating with discordant VL, immunologic and transcriptomic attributes, and clinical outcomes both pre and post ART.

Through evaluation of cohorts in which the estimated dates of infection or seroconversion could be computed, we found that, during primary and early HIV infection, 60% and 40% of HIV+ persons present with second-order CD8-CD4 equilibrium and advanced AIIS, respectively, and reflect an immunologic advantage and disadvantage, respectively (IDG-I or IDG-II vs. IHG-III or IDG-IV). By serial monitoring of IDGs and assessment of genetic status, we demonstrate that, at any time during the therapy-naïve disease course, persons classify into three major immunologic classes. First, preservation of immunologic advantage, despite high-grade HIV-associated antigenic stimulation; preservation is

attributable in part to carriage of beneficial alleles that restrict HIV entry/viral replication. Second, acquired immunologic disadvantage, attributable to a switch from second-order equilibrium to advanced AIIS before ART is initiated; this switch is more likely in persons bearing detrimental alleles. Third, intrinsic immunologic disadvantage, i.e., presentation with advanced AIIS, also attributable in part to carriage of detrimental alleles. While a switch from an immunologic advantage to an acquired disadvantage generally occurred one year after HIV seroconversion, it can develop at any time and may be preventable with early ART. Thus, from a practical standpoint, the restorative benefits of early ART are accrued mainly by patients preserving an immunologic advantage when ART is initiated.

We demonstrate that the IDGs but not the CD4+ count or CD4:CD8 T-cell ratio are a facile method to endotype HIV, pre and post ART. IDGs I to IV correlated with progressively (i) higher HIV VL and AIDS risks in therapy-naïve persons and (ii) constrained odds and pace of reconstituting AIIS-free status during ART irrespective of the interval between HIV acquisition and initiation of ART or the CD4+ count at ART initiation. Further underscoring that IDGs track unique endotypes, the nature of the IHG reconstituted during ART differed according to the IDG at ART initiation. Initiation of ART with IDG-I supported rapid reconstitution of IHG-I. IDG-II supported the rapid reconstitution of nearly equal proportions of IHG-I and IHG-II. IDG-III associated with a substantial delay in the reconstitution of IHG-I. IDG-IV associated with the worst reconstitution characteristics, as it supported mainly the reconstitution of IHG-III or IHG-IV and delayed reconstitution of IHG-I or IHG-II. Correspondingly, irrespective of the

timing and duration of ART, residual AIIS rates during long-term ART were least when initiating ART at IDG-I and maximal at IDG-IV.

This distinctive reconstitution pattern of IHGs and residual AIIS rates indexed to the IDG at ART initiation was in agreement with our finding that most serious adverse events were observed in individuals initiating ART in the presence of IDG-III or IDG-IV. Thus, decay rates of accumulated host antigenic burden required for reconstitution of AIIS-free status were progressively slower in persons initiating ART at IDG-I to IDG-IV. Hence, co-indexing the IDG at pre-ART with the IHG reconstitution patterns that emerge during ART may allow for precision HIV medicine: persons initiating ART at IDG-III or IDG-IV and demonstrating a delay in the reconstitution of IHG-I or IHG-II may benefit from more aggressive screening of AIDS and non-AIDS-related comorbidities.

Reconstitution of AIIS-free status is an important immunologic landmark to achieve, justifying this form of precision HIV medicine. First, at all ages, AIIS-free status is the most common state in HIV− persons. Second, reconstitution of AIIS-free status associated with several salutary traits in HIV+ persons, including lower IL-6 levels; near-normalization of some key proxies for T-cell health (T-cell responsiveness, activation, and dysfunction) and genomic metrics of immunosenescence (AIIS-1 score); superior vaccine responsiveness; and lower HIV DNA levels. The association of AIIS-free status with lower levels of IL-6 is notable, as it is a well-established biomarker that correlates with inflammation and age-associated diseases (*32, 33, 66-68*). The importance of normalizing AIIS genomic metrics is underscored by our observation that they correlated

negatively with a gene signature (**Figure S24**) that Alpert et al. showed predicted all-cause mortality more effectively than well-established cardiovascular risk factors in the Framingham Heart Study cohort (*69*).

Reversal of persistent AIIS despite immediate ART may require adjunctive immunotherapies that reduce the accumulated host antigenic burden and, consequently, comorbidities. However, this reversal may also require interventions focused on HIV risk factors. In our study, pre-existing AIIS was observed in 30% of FSWs who subsequently seroconverted and 38% of HIV– children with schistosomiasis (regardless of urine egg count). Some HIV– FSWs had advanced AIIS at baseline. Thus, chronic antigenic stimulation from varied non-HIV sources serves as a pathogenic driver of AIIS and is a shared feature of varied risk groups of HIV infection. Hence, our results and the unique restriction of HIV to risk groups support the following continuum model: at time of viral exposure, endemic AIIS in risk groups contributes to HIV acquisition, and after infection, the immunologic damage associated with pre-existing and HIV-induced AIIS may precipitate rapid progression to advanced AIIS, resulting in poor immunologic responses to ART. Since risk factor-associated AIIS is non-responsive to ART, its reversal requires mitigation of behavioral and nonbehavioral risk factors for HIV. Rates of syphilis and other STIs among HIV+ men who have sex with men have been rising (*15-17*). Thus, interventions to reduce behavioral risks that associate with induction of AIIS will have not only a positive public health impact but also benefit individual patients as an adjunctive method to reduce AIIS during ART.

In summary, our results support our hypothesis that (i) AIIS is a manifestation of unsuccessful immune allostasis, correlating with lower immunologic integrity at any age, and (ii) some age-associated diseases and acquisition of HIV and its sequelae may share a common etiological factor: persistent activation of the AIIS program. The possible deterministic role of CD8-CD4 disequilibrium states in potentiating adverse outcomes along the HIV acquisition/immune depletion/immune reconstitution continuum suggest a need to revisit the traditional framework of the drivers of HIV pathogenesis and outcomes. Despite receipt of optimal HIV care (early diagnosis, rapid ART at higher CD4+ counts, and maintenance of viral suppression), reconstitution of AIIS-free status may be substantially constrained in the subset of HIV+ persons who have a genetic predisposition to present with or progress to advanced AIIS. We provide a novel framework for precision HIV medicine and suggest that staging of HIV disease severity and immunologic reconstitution via CD8-CD4 equilibrium metrics have value as HIV cure strategies may be more beneficial in those with an immunologic advantage when initiating ART. Novel approaches to treating residual/endemic AIIS in persons with risk factors for HIV, with or without HIV infection, may need to be developed.

Methods

All studies were approved by the institutional review boards at the University of Texas Health Science Center at San Antonio and institutions participating in this study. Detailed methods included cohort characteristics are provided in Supplementary Methods.

Cohorts (*n,* Figure 1G)*.* Cohorts comprising children or adults with conditions correlated with varying types and grades of antigenic stimulation were evaluated. Six HIV–

cohorts/groups were evaluated (**Figure 1G**): (i) Adults in the SardiNIA cohort, established by a collaboration between National Institute on Aging and Sardinia to evaluate aging (*70*). (ii) FSWs who were HIV– at baseline and a subset (n=127) who seroconverted during prospective follow-up (**Table S2**) (*71*). To mitigate confounding of undiagnosed HIV among those with a single seronegative test for HIV antibodies, we focused on the subset (n=449) with at least two documented HIV seronegative antibody tests at least three months apart. (iii) Because schistosomiasis is a non-behavioral risk factor for acquiring HIV-1 (*20*), HIV– Kenyan children with or without *Schistosoma haematobium* ova in their urine were studied (*72*). (iv) RTRs evaluated for cancer risk (*50*). (v) Adults assessed for effects of recreational drug use at the UCSD. (vi) Adults accrued at UTHSCSA, San Antonio, TX.

Three HIV+ cohorts were evaluated: two were accrued during primary HIV infection (PIC) (*2, 73, 74*) and one during early HIV infection (EIC) (*3, 48*). The latter is the US Military HIV Natural History Study. PICs were from: (i) University of California San Diego (PIC-UCSD) (*2*) and (ii) Centre for the AIDS Programme of Research in South Africa (PIC-CAPRISA) (*73, 74*). Immunologic and virologic correlates of CD8-CD4 equilibrium were evaluated in subsets using methods described previously (*3, 54, 75, 76*). For individuals receiving ART, study duration was predefined as 10 years in the EIC (*3*) and 4 years in the PICs (*2*) from starting ART until failure of plasma HIV viral load (VL) suppression or cessation of ART.

Outcomes. In HIV– populations, outcomes were HIV seroconversion in FSWs and recurrent CSCC in RTRs. In therapy-naïve HIV+ persons, the outcomes were (i) anergy, reflected by the number of positive delayed-type hypersensitivity skin test reactions (*61*); (ii) higher HIV VL; (iii) development of AIDS (based on Centers for Disease Control and Prevention 1993 or 1987 criteria (*51, 52*)); and (iv) presentation or progression to AIIS or advanced AIIS.

For HIV+ persons receiving ART, level of reconstitution of immunologic integrity was benchmarked by two outcomes: (i) achieving first-order CD8-CD4 equilibrium (AIIS-free status), and (ii) development of severe clinical events, defined as non-AIDS-defining cancer or AIDS by the 1987 Centers for Disease Control and Prevention criteria. Immune correlates of immunologic integrity were also determined in HIV+ persons receiving long-term ART and in HIV– controls as described previously (*3, 54*), and included assessment of the integrity of the IL-7/IL-7 receptor axis, levels of T-cell activation and dysfunction, levels of plasma IL-6, and responsiveness to hepatitis B vaccine administered during ART (*3, 54, 75*).

Predictors. In HIV– persons, proxies were used to grade level of antigenic stimulation or exposures and served as predictors of host antigenic burden accumulated: (i) age was considered as a proxy for repetitive, low-grade antigenic experiences accrued during natural aging; (ii) a BAS based on behavioral risk factors (condom use, number of clients, number of condoms used per client) and a total STI score based on direct [syphilis (rapid plasma reagin test) and gonorrhea] and indirect (vaginal discharge, abdominal pain,

genital ulcer, dysuria, and vulvar itch) indicators of STI were used as proxies in HIV– FSWs for whom this information was available; and (iii) *S. haematobium* egg count in the urine was a proxy in children with this infection. For HIV+ persons, plasma HIV VL was a proxy for level of HIV-associated antigenic stimulation.

Predictors of outcomes in HIV– populations were the IHG at baseline, and in therapy-naïve HIV+ populations, the IHG or IDG at baseline and change in the IDG status from baseline during therapy-naïve disease course (IDG switch). Predictors of outcomes during ART were IDG before ART, interval between date of HIV acquisition and initiation of ART, and change in IDG grade between baseline and initiation of ART (IDG switch). To control for timing of ART, initiation was designated as earlier or later (*2, 3*). Earlier was defined as initiating ART within four months of estimated date of infection (EDI) in the PIC-UCSD and within 12 months of estimated date of seroconversion (EDS) in the EIC, as described previously (*2, 3*).

Genetic score. To determine whether host genetic factors predisposed to development of advanced AIIS during HIV infection, we derived a genetic score. Two polymorphisms in the gene encoding the major HIV coreceptor CC chemokine receptor 5 (CCR5) and four in the major histocompatibility complex locus were typed using standard allelic discrimination assays (*24, 61*). Genetic score was the number of beneficial alleles minus the number of the detrimental alleles (**Table S16**). The beneficial alleles were: rs333 (*CCR5*-Δ*32*) (*21*), rs4418214-T (in *MICA*) (*63*), rs9264942-T (in *HLA-C5'*) (*63*), and rs2395029-T (*HCP5*) (*62, 63*). The detrimental alleles were: rs2524054-A (in *HLA-B* and

HLA-*C5*') (*77*) and *CCR5* human haplotype E (*21*). Beneficial versus detrimental status relates to associations of the alleles with traits relevant to HIV/AIDS (HIV entry/replication, HIV VL, and AIDS progression rates) (*21, 23, 61-63, 77*) (**Table S16**). Scores ranged from –2 to 4, with higher scores reflecting a greater proportion of beneficial than detrimental alleles.

RNA-seq, bioinformatics methods and gene signature scores

RNA-Seq. RNA-seq was performed using samples from HIV+ and HIV– individuals (**Figures 5E,F**). All individual RNA-seq raw FASTQ files (2 x 101 bp, pair-ended) were aligned and mapped to the UCSC hg19 build of the *Homo sapiens sapiens* genome (from Illumina igenomes) using TopHat v2.0.1 (*78*). Counts for 23,239 unique, well-curated genes were obtained using HTSeq framework v0.5.3P3 (*79*).

Gene signature scores. To characterize and quantitate the genomic profile of AIIS, two gene signatures designated as AIIS-1 and AIIS-2 were derived (**Tables S12**). In post hoc analysis, signatures correlating with CD28– CD4+ or CD8+ T-cells and IFN-related processes were also derived (**Tables S12, S14**). The rationale for the derivation of the signatures is described in the Results section and calculations of the gene signature scores are shown in the Supplementary Methods.

Transcriptomic analyses. We evaluated the expression of gene signature scores in 15 publicly available microarray datasets from NCBI GEO and EMBL-EBI ArrayExpress (**Table S13**). Study designs for each dataset and methods of data normalization are

described in the Supplementary Methods. References for the gene expression datasets evaluated herein are available in **Table S13**.

Statistical analysis

Mann-Whitney U, chi-square, and Fisher's exact tests were used as appropriate. Cox proportional and logistic regression analyses were used to calculate hazard or rate ratios and odds ratios, respectively, with 95% CIs. CD4+ and CD8+ T-cell count trajectories (with 95% pointwise confidence bands) were derived using nonlinear generalized estimating equations. Incidence rate ratios were computed with a generalized linear model using quasi-Poisson distribution. Follow-up times and analyses were prespecified. Additional statistical methods used are described in the **Supplementary Appendix**.

AUTHOR CONTRIBUTIONS

SKA conceived the idea; designed, supervised, and coordinated the study; interpreted the data; and wrote the manuscript. JFO assembled datasets from the early HIV infection cohort for analysis, interpreted data and provided conceptual contributions. Additional cohorts and corresponding data were provided by LRM, MJB, SL, MS, EF, VO, FC, CK, JK, TBB, FAP, KRF, LW, NG, SSAK, S.G-W, DMS, SJL, DDR, TMF, and BKA. NH and WH performed statistical analysis. WH, JAM and MSM performed bioinformatics analyses. AB, AC, LP, KRC, and FJ provided experimental support. NH, WH, AB, MSM, JAM and SKA contributed to assembly of the online supplementary materials. NH, WH, AB, MSM, JAM, AMS, GCL, FJ, EAW, EJW, DDR, NZ, R.R-B, BKA, and RAC assisted in interpretation of data and provided editorial suggestions. The order of the co–second authors was determined by their relative contribution to this study. SKA and JFO obtained funding for the study and serve as joint senior authors.

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DISCLAIMER

The authors have no conflicts of interest to disclose. The views expressed are those of the authors and do not necessarily reflect the official views of the Uniformed Services University of the Health Sciences; the National Institutes of Health; the Department of Health and Human Services; Walter Reed National Military Medical Center; Brooke Army Medical Center; Madigan Army Medical Center; Naval Medical Center San Diego; Naval Medical Center Portsmouth; the Department of Defense; the Departments of the Army, Navy, or Air Force; or the Henry M. Jackson Foundation for the Advancement of Military Medicine. Mention of trade names, commercial products, or organizations does not imply endorsement by the U.S. government. The investigators have adhered to the policies for protection of human subjects as prescribed in 45 CFR 46. The U.S. government is authorized to reproduce and distribute reprints for governmental purposes notwithstanding any copyright notation thereon.

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Figure Legends

Figure 1. Study hypothesis/models, CD8-CD4 equilibrium metrics and cohorts. (**A**) Model linking antigenic (Ag) stimulation, immune allostasis, age-independent immunosenescence (AIIS) and immunologic integrity to outcomes. (**B**) Inducers and age of onset of AIIS. (**C**) Two sources of antigenic stimulation contribute to AIIS in HIV+ persons; AIIS preceding HIV infection is unresponsive to virally suppressive antiretroviral therapy (ART). (**D**) Successful immune allostasis following antigenic (Ag) stimulation associates with equilbirum between CD8+ and CD4+ T-cell levels quantifiable by first-order CD8-CD4 equilibrium metrics [immune health (IHG) grades]. (**E**) Progression from first-order to second-order equilibrium/disequilibrium states quantifiable by CD8-CD4 equilibrium metrics [immune health (IHG) and damage (IDG) grades]. Polarities of immunologic integrity and accumulated host antigenic (Ag) burden are depicted. (**F**) CD8:CD4 equilibrium metrics (IHGs and IDGs) derived by co-indexing the CD4:CD8 T-cell ratio and CD4+ T-cell counts (cells/mm^3) at the indicated cutoff levels. IHGs and IDGs track first-order and second-order CD8-CD4 equilibrium/disequilibrium states, respectively. (**G**) Cohorts and gene expression datasets studied.

Figure 1

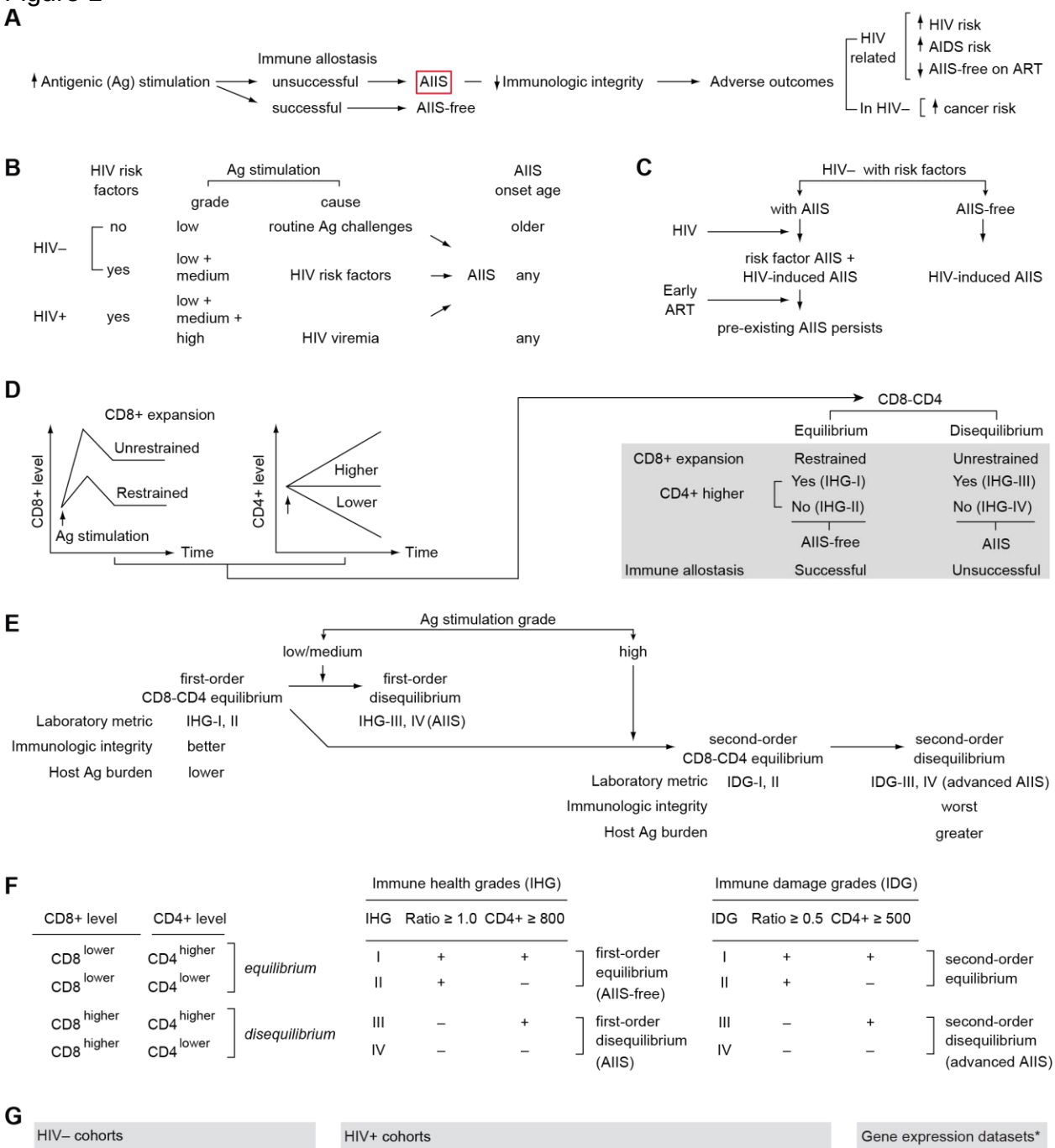
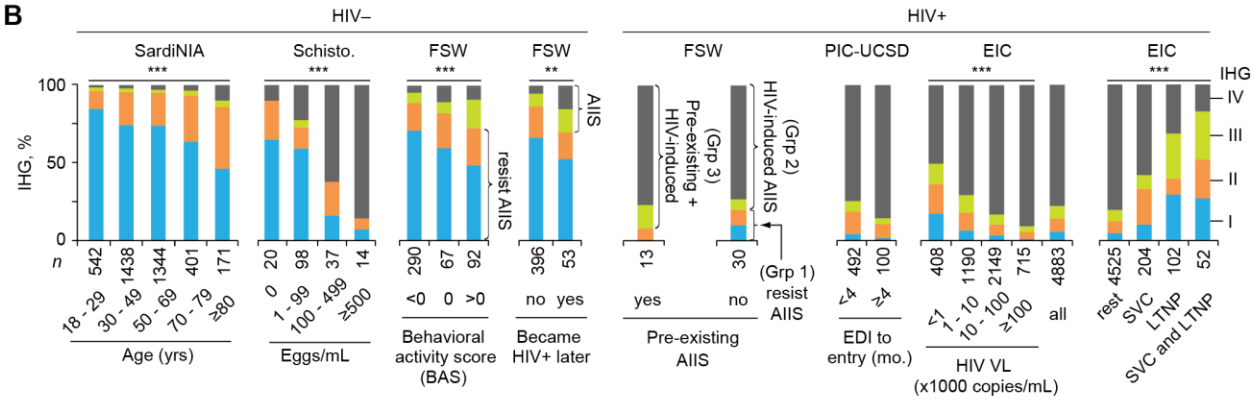

A
Antigenic (Ag) stimulation
Immune allostasis
unsuccessful
successful
AIIS
AIIS-free
Immunologic integrity
Adverse outcomes
HIV related
HIV risk
AIDS risk
AIIS-free on ART
In HIV–
cancer risk
B
HIV risk factors
Ag stimulation
grade
cause
AIIS onset age
HIV–
no
yes
low
routine Ag challenges
older
low + medium
HIV risk factors
AIIS
any
HIV+
yes
low + medium + high
HIV viremia
any
C
HIV– with risk factors
with AIIS
AIIS-free
HIV
risk factor AIIS + HIV-induced AIIS
HIV-induced AIIS
Early ART
pre-existing AIIS persists
D
CD8+ expansion
Unrestrained
Restrained
CD8+ level
Ag stimulation
Time
CD4+ level
Higher
Lower
Time
CD8-CD4
Equilibrium
Disequilibrium
CD8+ expansion
Restrained
Unrestrained
CD4+ higher
Yes (IHG-I)
No (IHG-II)
Yes (IHG-III)
No (IHG-IV)
AIIS-free
AIIS
Immune allostasis
Successful
Unsuccessful
E
Ag stimulation grade
low/medium
high
first-order CD8-CD4 equilibrium
first-order disequilibrium
Laboratory metric
Immunologic integrity
Host Ag burden
IHG-I, II
better
lower
IHG-III, IV (AIIS)
second-order CD8-CD4 equilibrium
second-order disequilibrium
Laboratory metric
Immunologic integrity
Host Ag burden
IDG-I, II
IDG-III, IV (advanced AIIS)
worst
greater
F
CD8+ level
CD4+ level
CD8 lower
CD4 higher
CD8 lower
CD4 lower
equilibrium
CD8 higher
CD4 higher
CD8 higher
CD4 lower
disequilibrium
Immune health grades (IHG)
IHG
Ratio ≥ 1.0
CD4+ ≥ 800
I
II
III
IV
first-order equilibrium (AIIS-free)
first-order disequilibrium (AIIS)
Immune damage grades (IDG)
IDG
Ratio ≥ 0.5
CD4+ ≥ 500
I
II
III
IV
second-order equilibrium
second-order disequilibrium (advanced AIIS)
G
HIV– cohorts
Low risk SardiNIA, n = 3896
Female sex workers, n = 1050
Children with schistosomiasis, n = 169
Renal transplant recipients, n = 114
HIV– UCSD controls, n = 759
HIV– UTHSCSA controls, n = 13
HIV+ cohorts
Primary Infection Cohort (PIC) with estimated date of infection:
PIC-UCSD, n = 685
PIC-CAPRISA, n = 99
Early Infection Cohort (EIC) from U.S. Natural History Study, n = 4883
Participants with estimated date of seroconversion, n = 3150
HIV+ spontaneous virologic controllers (UTHSCSA), n =31
Gene expression datasets*
HIV–, n = 3826
HIV+, n = 638
*, no. of samples

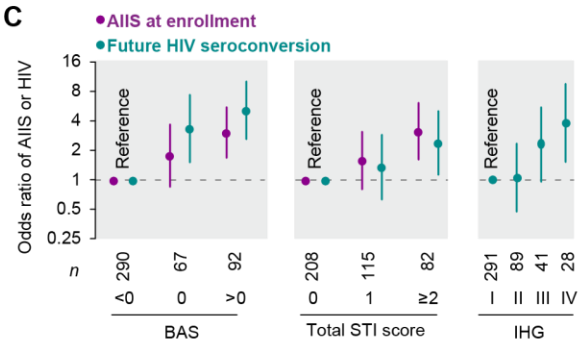
Figure 2. Immune health grade (IHG), accumulated host antigenic burden and outcomes. (**A**) The *n* values (%), median (IQR) CD8+ and CD4+ T-cell counts (cells/mm^3), and CD4:CD8 ratio by immune health grade (IHG) in HIV− University of California San Diego (UCSD) cohort. AIIS, age-independent immunosenescence. (**B**) Distribution of IHGs in HIV− and HIV+ cohorts. HIV− cohorts (left to right): SardiNIA cohort stratified by age; children with schistosomiasis (schisto.) stratified by urine egg count of *S. haematobium*; female sex workers (FSWs) stratified first by baseline behavioral activity score (BAS) and then by subsequent HIV seroconversion status. HIV+ cohorts (left to right): HIV+ FSWs stratified by pre- and post-infection IHG status; participants of the primary infection cohort (PIC) cohort from UCSD (PIC-UCSD) stratified by duration of untreated infection indexed to the estimated date of infection (EDI) in months (mo.); and participants of the early infection cohort (EIC) stratified by plasma viral load (VL, copies/ml) at entry as well as by whether participants classified as spontaneous virologic controllers (SVC) or long-term non-progressors (LTNP). *P,* χ^2 test except Fisher's exact test for schistosomiasis data. (**C**) Odds of having AIIS at baseline and future HIV seroconversion in FSWs according to baseline BAS, total sexually transmitted infection (STI) scores, and IHGs. Odds ratios are depicted with 95% confidence intervals. (**D**) Kaplan-Meier plots showing time to AIIS by baseline BAS (left) and total STI scores (right) in FSWs that were AIIS-free at baseline and remained HIV−. *P*, log-rank test. (**E**) Distribution of IHGs at baseline (overall and by IHG) and at first available IHG after 4 years in 101 FSWs that remained HIV− for at least 4 years. Half-moon arrows signify change in IHG distribution from baseline. (**F**) Distribution of IHGs at enrollment in HIV− renal transplant recipients (RTRs). (**G**) Time to second occurrence of cutaneous squamous cell carcinoma (CSCC) by IHG at enrollment in RTRs. *P*, log-rank test. (**H**) Schema for ranking accumulated host antigenic burden according to distribution of IHGs in indicated study populations.

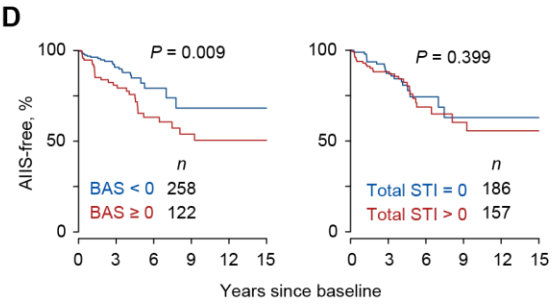
Figure 2

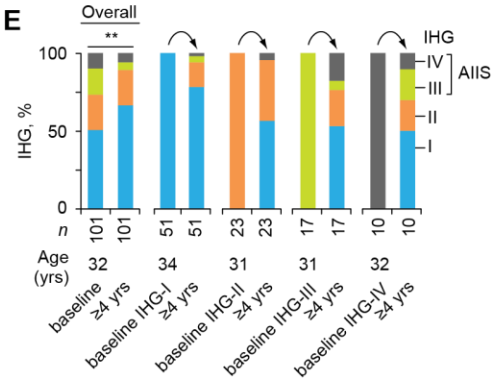
A

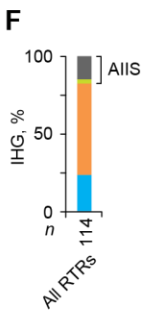
IHG	*n* (%)	CD8+	CD4+	Ratio	CD8+ expansion	CD8+	CD4+	Equilibrium	AIIS status
I	492 (64.8)	556 (420-703)	1063 (917-1248)	1.92 (1.53-2.53)	Restrained	Lower	Highest	first-order equilibrium	AIIS-free
II	223 (29.4)	391 (290-498)	674 (591-739)	1.64 (1.37-2.13)	Suppressed	Lowest	Lower		
III	19 (2.5)	1054 (948-1187)	930 (902-1017)	0.89 (0.87-0.97)	Unrestrained	Highest	Higher	first-order disequilibrium	AIIS
IV	25 (3.3)	715 (645-833)	582 (539-706)	0.89 (0.72-0.91)	Unrestrained	Higher	Lowest		

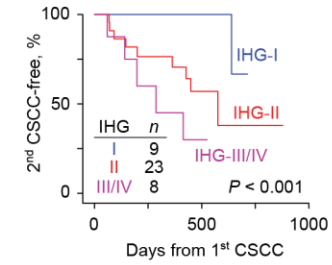












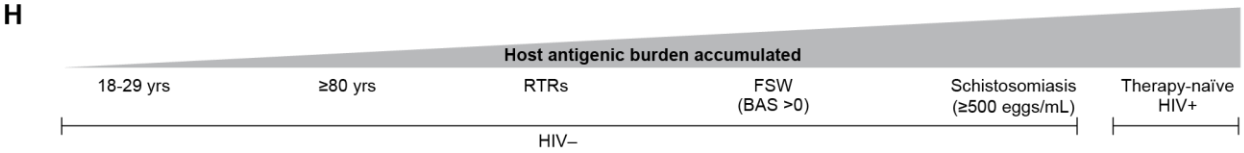


Figure 3. Immune damage grade (IDG) and outcomes in therapy-naïve HIV+ persons from the early infection cohort (EIC). (**A**) Schema depicting rapid post-infection binning of HIV+ persons into those who did vs. did not preserve first-order equilibrium states into second-order equilibrium (IDG-I or IDG-II) and disequilibrium (IDG-III or IDG-IV) states. Schema summarizes data from Figure 2B and 3B,C without consideration of pre-existing AIIS status. (**B**) IDGs at entry according to interval between study entry (baseline) and estimated date of infection (EDI) or seroconversion (EDS) in primary infection (PIC-UCSD) and early infection (EIC) cohorts, respectively. (**C**) Prevalence of IDGs in EIC participants according to the IHG at entry (left) and during four years of therapy-naïve disease course (right). Triangle, proportion of switching to advanced AIIS by IHG at entry. (**D**) Unadjusted odds ratio and 95% confidence intervals of having advanced AIIS in year 4 post entry by IHG at entry. (**E**) Kaplan-Meier plots of time to AIDS (CDC 1993 criteria) by IHG (left) or IDG (right) status at entry; also depicted are entry median CD4+ and CD8+ T-cell counts (cells/mm^3) and median CD4:CD8 T-cell ratio, and median $\log_{10}$ viral load (VL, copies/ml) for each grade. *P*, log-rank test. VL*******, *P*<0.001 difference from IHG-I or IDG-I determined by linear regression. (**F**) Time to AIDS (CDC 1993 criteria) by three strata of entry CD4+ T-cell counts (cells/mm^3), before (leftmost; blinded to IDG) and after accounting for entry IDG status. *P*, log-rank test.

Figure 3

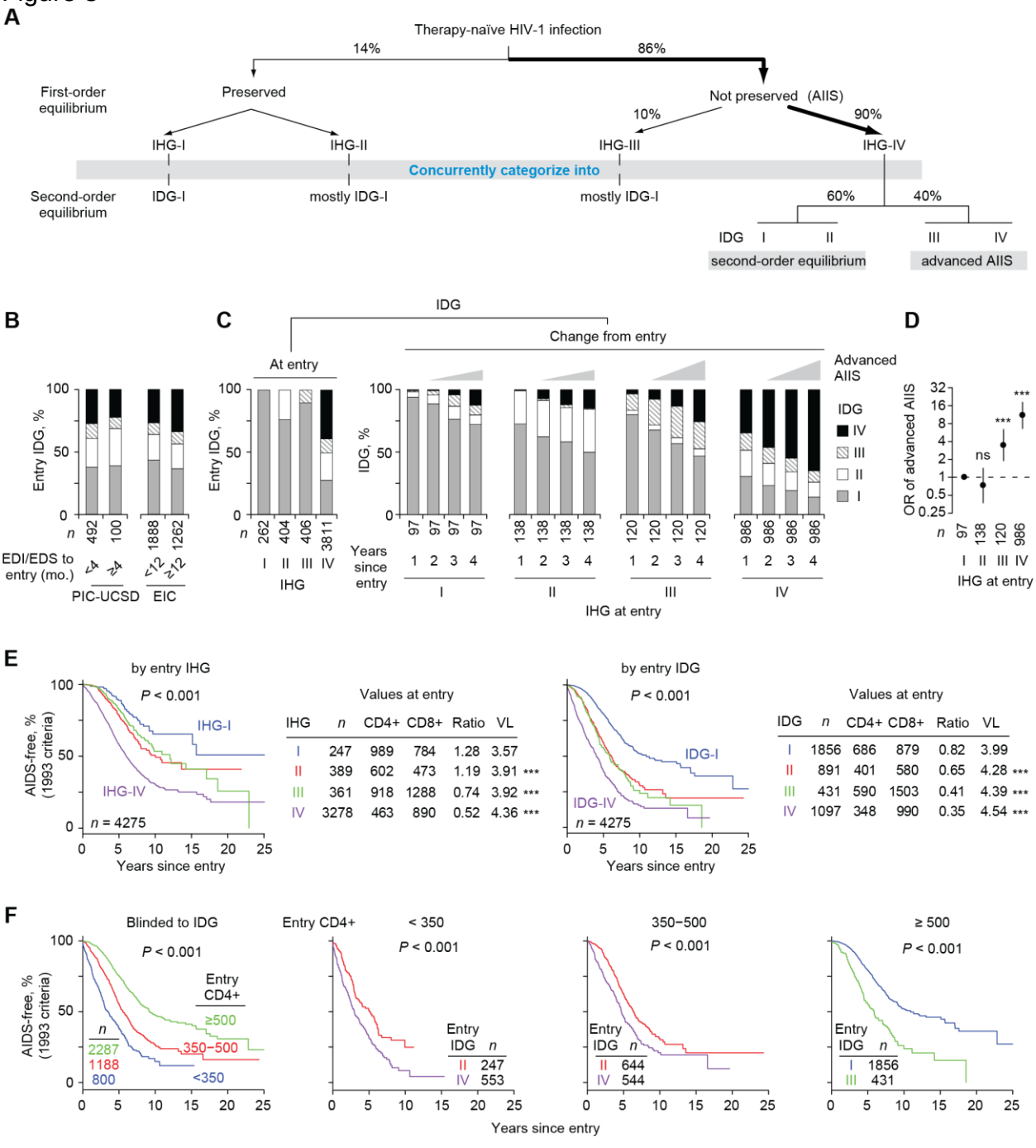

A
Therapy-naïve HIV-1 infection
14%
86%
First-order equilibrium
Preserved
Not preserved (AIIS)
10%
90%
IHG-I
IHG-II
IHG-III
IHG-IV
Concurrently categorize into
Second-order equilibrium
IDG-I
mostly IDG-I
mostly IDG-I
60%
40%
IDG I II
III IV
second-order equilibrium
advanced AIIS
B
Entry IDG, %
n 492 100 1888 1262
EDI/EDS to entry (mo.) <4 ≥4 <12 ≥12
PIC-UCSD EIC
C
IDG
At entry
Change from entry
Advanced AIIS
IDG IV III II I
Entry IDG, %
n 262 404 406 3811
IHG I II III IV
IDG, %
Years since entry
1 2 3 4
IHG at entry
D
OR of advanced AIIS
n 97 138 120 986
ns
IHG at entry
E
by entry IHG
P < 0.001
IHG-I
IHG-IV
n = 4275
AIDS-free, % (1993 criteria)
Years since entry
Values at entry
IHG n CD4+ CD8+ Ratio VL
I 247 989 784 1.28 3.57
II 389 602 473 1.19 3.91 ***
III 361 918 1288 0.74 3.92 ***
IV 3278 463 890 0.52 4.36 ***
by entry IDG
P < 0.001
IDG-I
IDG-IV
n = 4275
Years since entry
Values at entry
IDG n CD4+ CD8+ Ratio VL
I 1856 686 879 0.82 3.99
II 891 401 580 0.65 4.28 ***
III 431 590 1503 0.41 4.39 ***
IV 1097 348 990 0.35 4.54 ***
F
Blinded to IDG
P < 0.001
Entry CD4+
n
2287 ≥500
1188 350–500
800 <350
AIDS-free, % (1993 criteria)
Entry CD4+ < 350
P < 0.001
Entry IDG n
II 247
IV 553
350–500
P < 0.001
Entry IDG n
II 644
IV 544
≥ 500
P < 0.001
Entry IDG n
I 1856
III 431
Years since entry

Figure 4. Immune damage grade (IDG) switching and HIV disease endotypes in therapy-naïve HIV+ persons from the early infection cohort (EIC). (A) Top, schema depicting IDG switching and immunosuppression status during therapy-naïve disease course. Bottom, Kaplan-Meier plots of time to AIDS (CDC 1993 criteria) according to whether a switch in IDG had occurred between entry and the third year of therapy-naïve disease course. *P*, log rank test. (**B**) HIV disease endotypes. Top, trajectories of CD4+ (blue) and CD8+ (red) counts and HIV viral load (VL) (green) with 95% pointwise confidence bands of persons presenting with IDG-I, II, III, or IV, before (first column; blinded to IDG switch) and after accounting for whether a change in IDG status occurred between entry and third year of disease course without virally-suppressive antiretroviral therapy. Bottom, schema outlining attributes and outcomes associated with the HIV endotypes defined by the IDGs.

Figure 4

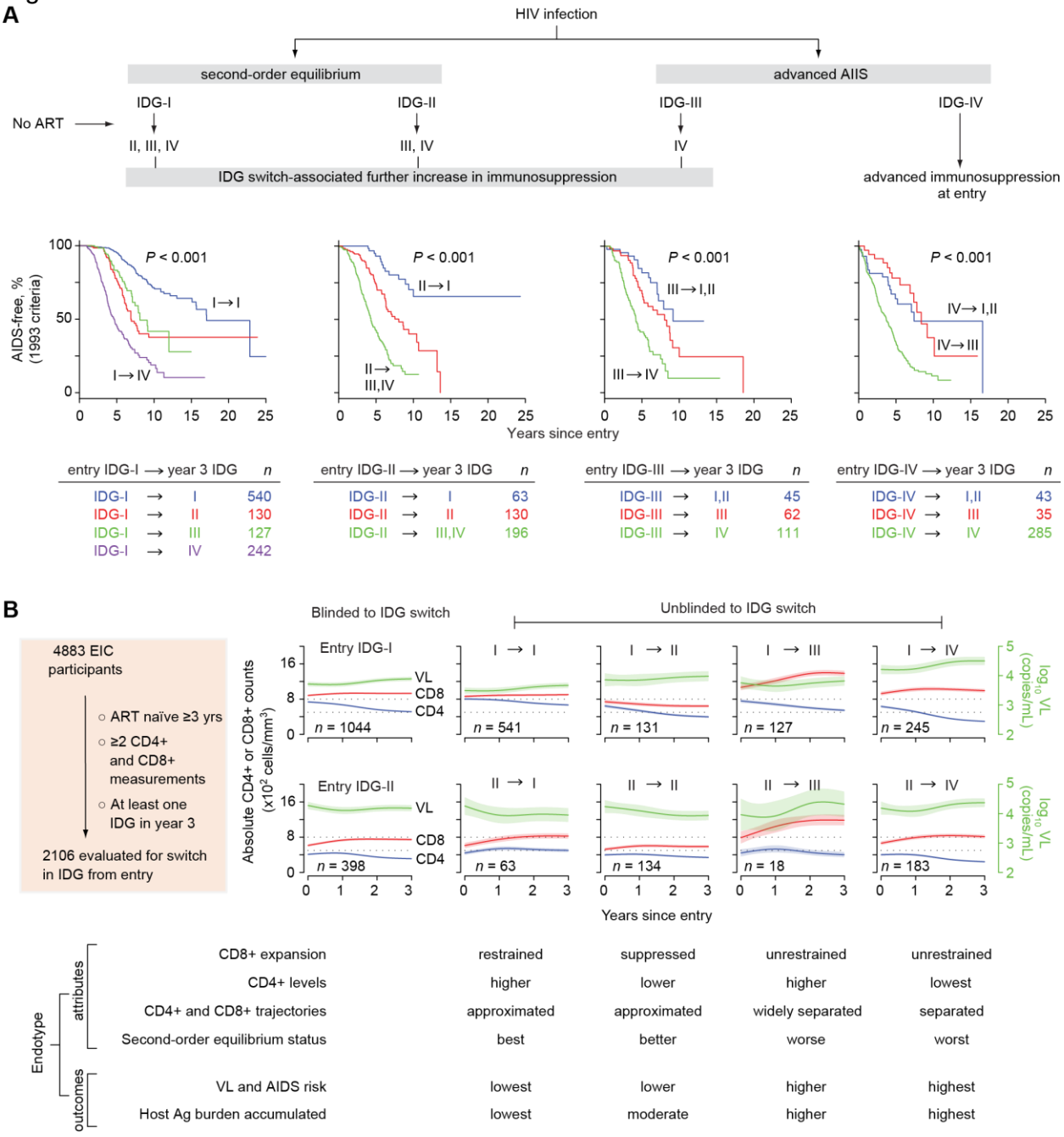

A
HIV infection
second-order equilibrium
advanced AIIS
No ART
IDG-I
II, III, IV
IDG-II
III, IV
IDG-III
IV
IDG-IV
IDG switch-associated further increase in immunosuppression
advanced immunosuppression at entry
AIDS-free, % (1993 criteria)
P < 0.001
I→I
I→IV
II→I
II→ III,IV
III→I,II
III→IV
IV→I,II
IV→III
Years since entry
entry IDG-I → year 3 IDG n
IDG-I → I 540
IDG-I → II 130
IDG-I → III 127
IDG-I → IV 242
entry IDG-II → year 3 IDG n
IDG-II → I 63
IDG-II → II 130
IDG-II → III,IV 196
entry IDG-III → year 3 IDG n
IDG-III → I,II 45
IDG-III → III 62
IDG-III → IV 111
entry IDG-IV → year 3 IDG n
IDG-IV → I,II 43
IDG-IV → III 35
IDG-IV → IV 285
B
4883 EIC participants
ART naïve ≥3 yrs
≥2 CD4+ and CD8+ measurements
At least one IDG in year 3
2106 evaluated for switch in IDG from entry
Blinded to IDG switch
Unblinded to IDG switch
Entry IDG-I
Entry IDG-II
I → I
I → II
I → III
I → IV
II → I
II → II
II → III
II → IV
VL
CD8
CD4
Absolute CD4+ or CD8+ counts (x10² cells/mm³)
log₁₀ VL (copies/mL)
n = 1044
n = 541
n = 131
n = 127
n = 245
n = 398
n = 63
n = 134
n = 18
n = 183
Years since entry
Endotype
attributes
outcomes
CD8+ expansion restrained suppressed unrestrained unrestrained
CD4+ levels higher lower higher lowest
CD4+ and CD8+ trajectories approximated approximated widely separated separated
Second-order equilibrium status best better worse worst
VL and AIDS risk lowest lower higher highest
Host Ag burden accumulated lowest moderate higher highest

Figure 5. **Immunologic and genomic (gene expression) attributes/traits of the age-independent immunosenescence (AIIS) program.** (**A**) Associations between immune health grade (IHG) reconstituted during long-term virally-suppressive antiretroviral therapy (ART) with indicated immune traits in 8 groups (Grp.) categorized by IHG, cytomegalovirus (CMV) serostatus, ART status, viremic status, and HIV serostatus. HIV+ persons are from the early infection cohort (EIC). +/−, yes/no. *P*, Wilcoxon rank sum test. ****P*<0.001. (**B**) Confounding by conflation when comparing immune traits in individuals according to whether the CD4+ count achieved during ART was ≥800 or <800 cells/mm^3 rather than by IHG status as shown in panel A. Group numbers refer to the groups in panel A. (**C**) Plasma IL-6 levels by AIIS and CMV serostatus in EIC participants. *P*, Wilcoxon rank sum test. **P*<0.05. (**D**) Proportion of HIV+ participants from the EIC responsive to hepatitis B virus (HBV) vaccine administered during virally suppressive ART. Anti-HbS, hepatitis B surface antibody. *P,* χ^2 test. **P*<0.05. (**E,F**) AIIS-1 (E) and AIIS-2 (F) gene signature scores in EIC participants categorized as in panel A, except for the inclusion of spontaneous virologic controllers (SVC) instead of Group 7 in panel A. Panels (**G-O**) AIIS-2 gene signature score in the following cohorts/samples. Unless otherwise indicated cohorts/samples are from HIV− persons. (**G**) Whole blood samples from 36 recently HIV-infected patients before and after 48 weeks of ART. (Source: GSE44228). *P*, ANOVA of GEE (generalized estimating equation) model. (**H**) CD4+ T-cell samples from HIV+ patients stratified by surface PD (programmed cell death)-1 expression, HIV viral load (VL; k, x 10^3 copies/mL) and peripheral blood CD4+ T-cell count (Source: GSE17606). *P*, Kruskal-Wallis Test for overall comparison of the groups. (**I**) Meta-analysis of gene expression data sets from whole blood of HIV− and HIV+ persons from the indicated geographical locations and conditions. HIV− persons were otherwise healthy or had latent tuberculosis infection (LTBI) or pulmonary tuberculosis (PTB). ART status (yes/no) of HIV+ persons is indicated. Horizontal dashed line and grey band: IQR of score in HIV− healthy older persons (65-74 years) from the Finnish cohort. Representative cohorts from Finland, U.S.A., Africa and U.K. are shown here and the complete panel is shown in Figure S14 (Sources: Meta-analysis of E-TABM-1036, GSE29429, GSE19435, GSE19439, GSE19442, GSE19444). See Supplemental Materials for detailed statistical methods used. (**J**) Lymphocyte samples from 1,240 Mexican-Americans by age (Source: E-TABM-305). *P*, Kruskal-Wallis Test for overall comparison of the groups. (**K**) Peripheral blood mononuclear cell samples from pre and post Epstein-Barr virus (EBV) infection (acute infectious mononucleosis) (Source: GSE45918). *P*, Wilcoxon signed-rank test for paired comparison of the groups. (**L**) Whole blood samples from 133 participants at enrollment (baseline) and followed longitudinally during seasonal acute respiratory illness (days 0 [within 48hr of onset of fever], 2, 4, 6, 21) and during the spring of the next year (Source: GSE68310). *P*, ANOVA of GEE model. (**M**) Blood samples from healthy controls (Ctrl.) and RSV-infected infants at acute and recovery timepoints, stratified by disease severity (mild, moderate, and severe) (Source: E-MTAB-5195). *P*, GEE model between acute and recovery timepoint groups adjusting for severity. (**N**) CD4+ T-cell samples from healthy controls (Ctrl.) and HIV− persons with sarcoidosis (SARC) and idiopathic CD4+ lymphocytopenia (ICL) (Source: GSE56998). *P*, Kruskal-Wallis test for overall comparison of the groups. (**O**) Whole blood samples from 18 asymptomatic ART-treated HIV+ patients who volunteered for an analytical treatment interruption (ATI) at week 24 after receipt of a dendritic cell-based vaccine at the indicated

timepoints (Source: GSE46734). *P*, GEE model between indicated timepoints. ns, non-significant. ***P*<0.01, ****P*<0.001.

Figure 5

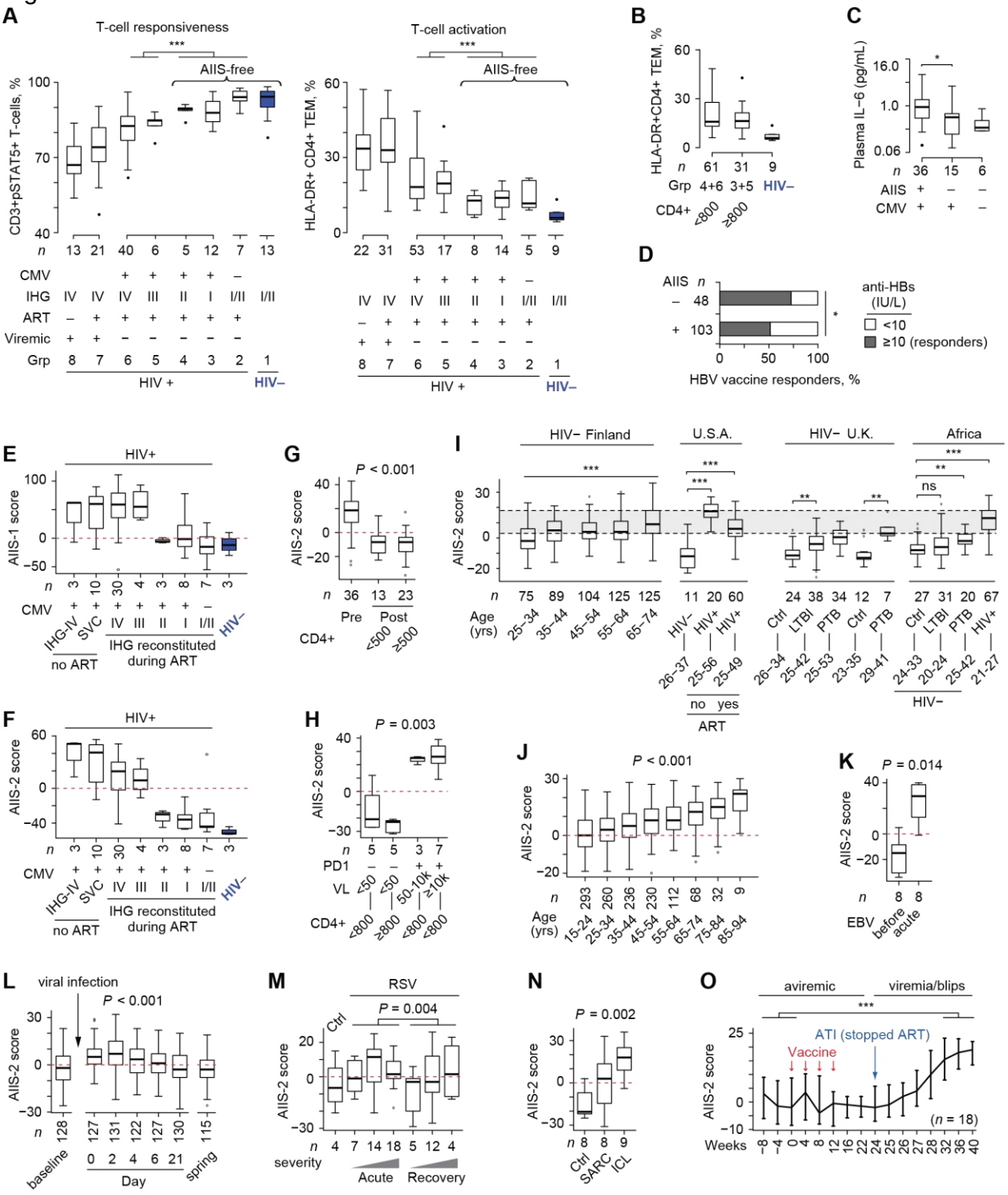


Figure 6. **Reconstitution of age-independent immunosenescence (AIIS)-free status and severe clinical events during antiretroviral therapy (ART) conditional on immune damage grade (IDG) at initiation of virally suppressive ART.** (**A**) Kaplan-Meier plots depicting rate ratios (RR) of achieving AIIS-free status in participants of the primary infection cohort of University of California San Diego (PIC-UCSD), early HIV infection cohort (EIC), and PIC-Centre for the AIDS Programme of Research in South Africa (CAPRISA) cohorts according to IDG at ART initiation. The interval [in median (IQR) months (mo.)] between EDI (estimated date of infection for PICs) and EDS (estimated date of seroconversion for EIC) and ART initiation is depicted on the top. (**B**) Hazard of developing a new serious clinical event (e.g., cancer) in EIC participants initiating ART by pre-ART IDG. Depicted below the plots in panels A and B are the pre-ART CD4+ counts (cells/mm^3), rate ratios (RRs) or hazard ratios (HRs) with 95% confidence intervals by pre-ART IDG, The rate ratio and hazard ratio (aRR and aHR) in the EIC are adjusted for calendar year of ART initiation, ART regimen, age, sex, and ethnicity. The IDG at pre-ART was derived by using laboratory values determined within 6 months of starting ART. (**C**) Kaplan-Meier plots depicting RR for achieving AIIS-free status in persons presenting with CD4+ counts ≥500 cells/mm^3, but initiating ART at CD4+ <350 (deferred-initiation arm), 350-500 (intermediate-initiation arm), and ≥500 (immediate-initiation arm) cells/mm^3; these groups were further stratified by IDG at pre-ART. The immediate- and deferred-initiation arms mirror the arms of the randomization strategy of the START clinical trial (*44*). *P*, log-rank test. (**D**) Left to right for the indicated study groups of the EIC derived according to the START randomization strategy, before and after accounting for the IDG when ART was initiated: median values of laboratory measures, odds of achieving AIIS-free status, and IHGs reconstituted during ART. The best IHG achieved during ART was used in the stacked bar plots. *P*, χ^2 test. aOR, adjusted odds ratio (adjusted for ART regimen, age, sex, and ethnicity). ***P<0.001.

Figure 6

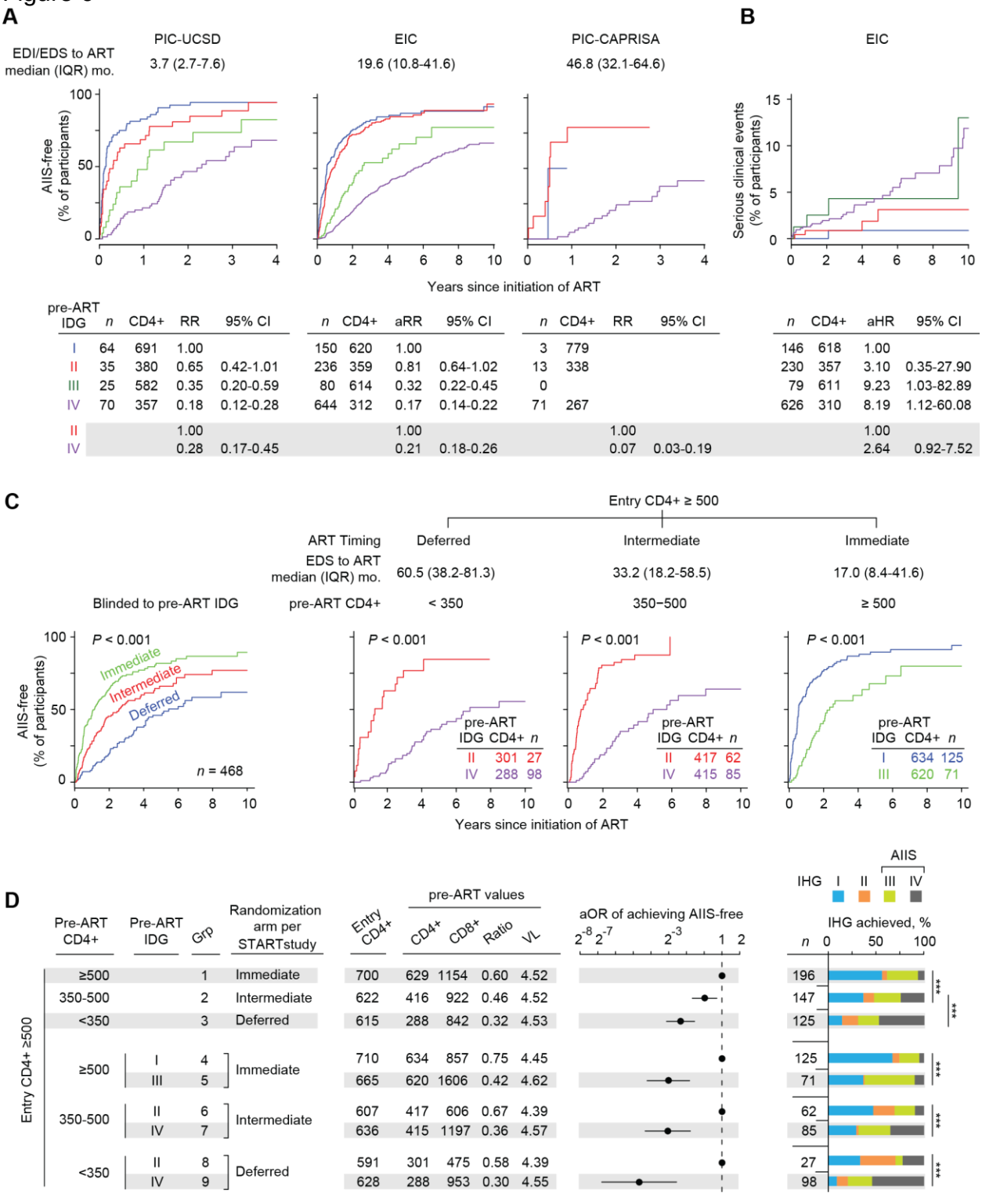

A
PIC-UCSD
EIC
PIC-CAPRISA
EDI/EDS to ART median (IQR) mo.
3.7 (2.7-7.6)
19.6 (10.8-41.6)
46.8 (32.1-64.6)
AIIS-free (% of participants)
Years since initiation of ART
B
EIC
Serious clinical events (% of participants)
pre-ART IDG | n | CD4+ | RR | 95% CI | n | CD4+ | aRR | 95% CI | n | CD4+ | RR | 95% CI | n | CD4+ | aHR | 95% CI
I | 64 | 691 | 1.00 | | 150 | 620 | 1.00 | | 3 | 779 | | | 146 | 618 | 1.00 |
II | 35 | 380 | 0.65 | 0.42-1.01 | 236 | 359 | 0.81 | 0.64-1.02 | 13 | 338 | | | 230 | 357 | 3.10 | 0.35-27.90
III | 25 | 582 | 0.35 | 0.20-0.59 | 80 | 614 | 0.32 | 0.22-0.45 | 0 | | | | 79 | 611 | 9.23 | 1.03-82.89
IV | 70 | 357 | 0.18 | 0.12-0.28 | 644 | 312 | 0.17 | 0.14-0.22 | 71 | 267 | | | 626 | 310 | 8.19 | 1.12-60.08
II | | | 1.00 | | | | 1.00 | | | | 1.00 | | | | 1.00 |
IV | | | 0.28 | 0.17-0.45 | | | 0.21 | 0.18-0.26 | | | 0.07 | 0.03-0.19 | | | 2.64 | 0.92-7.52
C
Entry CD4+ ≥ 500
ART Timing
Deferred
Intermediate
Immediate
EDS to ART median (IQR) mo.
60.5 (38.2-81.3)
33.2 (18.2-58.5)
17.0 (8.4-41.6)
Blinded to pre-ART IDG
pre-ART CD4+
< 350
350–500
≥ 500
P < 0.001
Immediate
Intermediate
Deferred
n = 468
pre-ART IDG CD4+ n
II 301 27
IV 288 98
II 417 62
IV 415 85
I 634 125
III 620 71
Years since initiation of ART
D
Pre-ART CD4+
Pre-ART IDG
Grp
Randomization arm per STARTstudy
Entry CD4+
pre-ART values
CD4+
CD8+
Ratio
VL
aOR of achieving AIIS-free
IHG
AIIS
IHG achieved, %
Entry CD4+ ≥500
≥500 | | 1 | Immediate | 700 | 629 | 1154 | 0.60 | 4.52 | 196
350-500 | | 2 | Intermediate | 622 | 416 | 922 | 0.46 | 4.52 | 147
<350 | | 3 | Deferred | 615 | 288 | 842 | 0.32 | 4.53 | 125
≥500 | I | 4 | Immediate | 710 | 634 | 857 | 0.75 | 4.45 | 125
≥500 | III | 5 | Immediate | 665 | 620 | 1606 | 0.42 | 4.62 | 71
350-500 | II | 6 | Intermediate | 607 | 417 | 606 | 0.67 | 4.39 | 62
350-500 | IV | 7 | Intermediate | 636 | 415 | 1197 | 0.36 | 4.57 | 85
<350 | II | 8 | Deferred | 591 | 301 | 475 | 0.58 | 4.39 | 27
<350 | IV | 9 | Deferred | 628 | 288 | 953 | 0.30 | 4.55 | 98

Figure 7. Immune health grade (IHG) reconstituted during virally-suppressive antiretroviral therapy (ART) is conditional on immune damage grade (IDG) at ART initiation. (**A**) IHGs reconstituted during long-term ART in the early infection cohort (EIC) and primary infection cohort of University of California San Diego (PIC-UCSD) participants. Top and bottom represent EIC and PIC-UCSD participants, respectively, overall and in those initiating ART earlier and later (within or after 12 months of the estimated date of seroconversion in the EIC and within or after 4 months since estimated date of infection in the PIC-UCSD). *P*, χ^2 test. (**B**) IHG reconstituted by IDG at pre-ART in EIC participants who initiated ART earlier and PIC-UCSD participants. Best IHG achieved during ART in the indicated time windows post-ART are depicted in the stacked bar plots. To rank residual AIIS and host antigenic burden, the distribution of IHGs in the indicated HIV− populations are shown. (**C**) Model depicting that post-ART residual AIIS (a marker of increased accumulated host antigenic burden) is conditional on the IDG at pre-ART. Model ranks pre-ART (according to Figure 4B) and post-ART (according to Figure 2H) accumulated host antigenic burden. Ranking of residual host antigenic burden post-ART was based on comparisons of the IHG distributions in HIV+ persons vs indicated HIV− study groups in panel B.

Figure 7

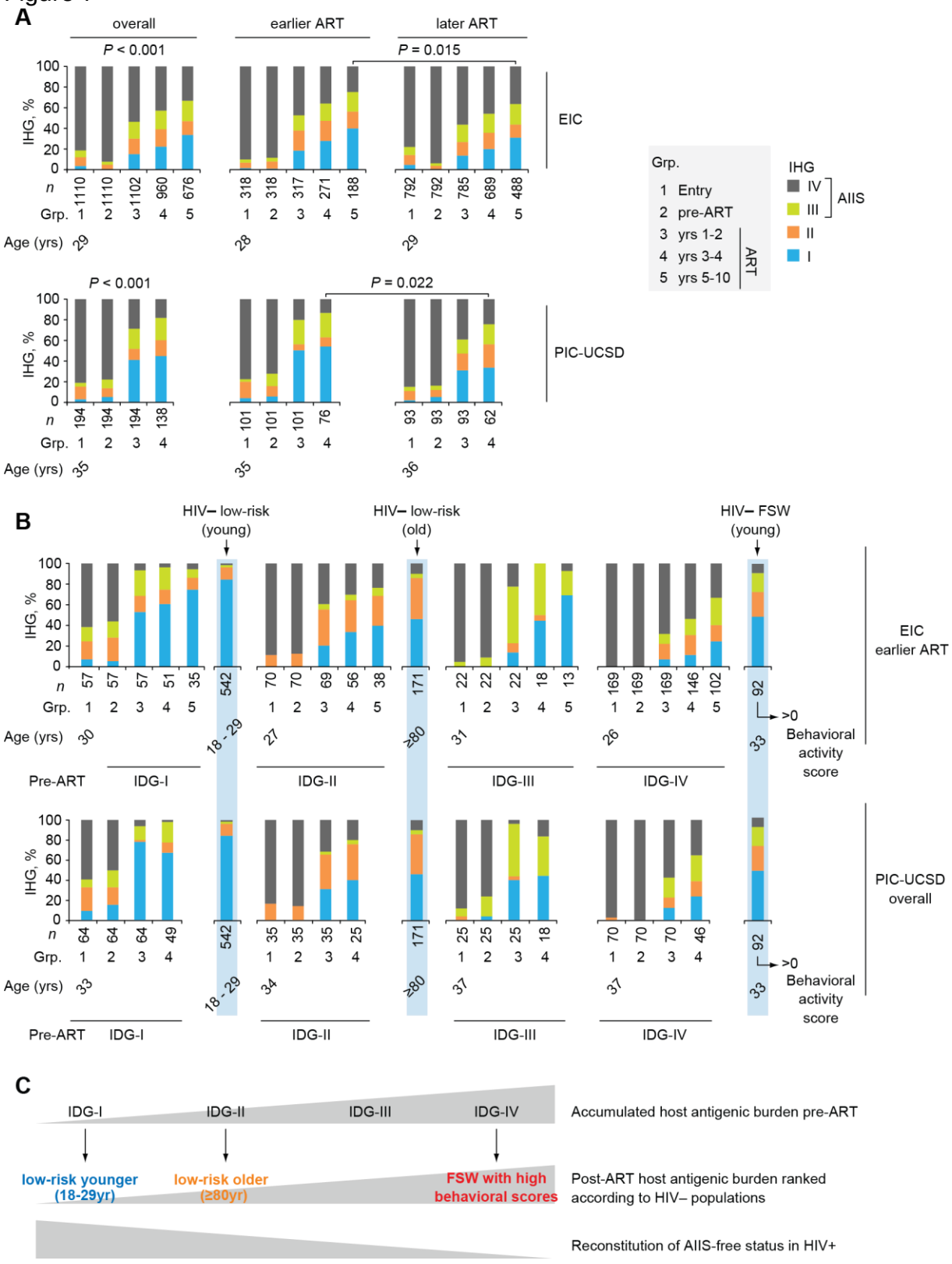
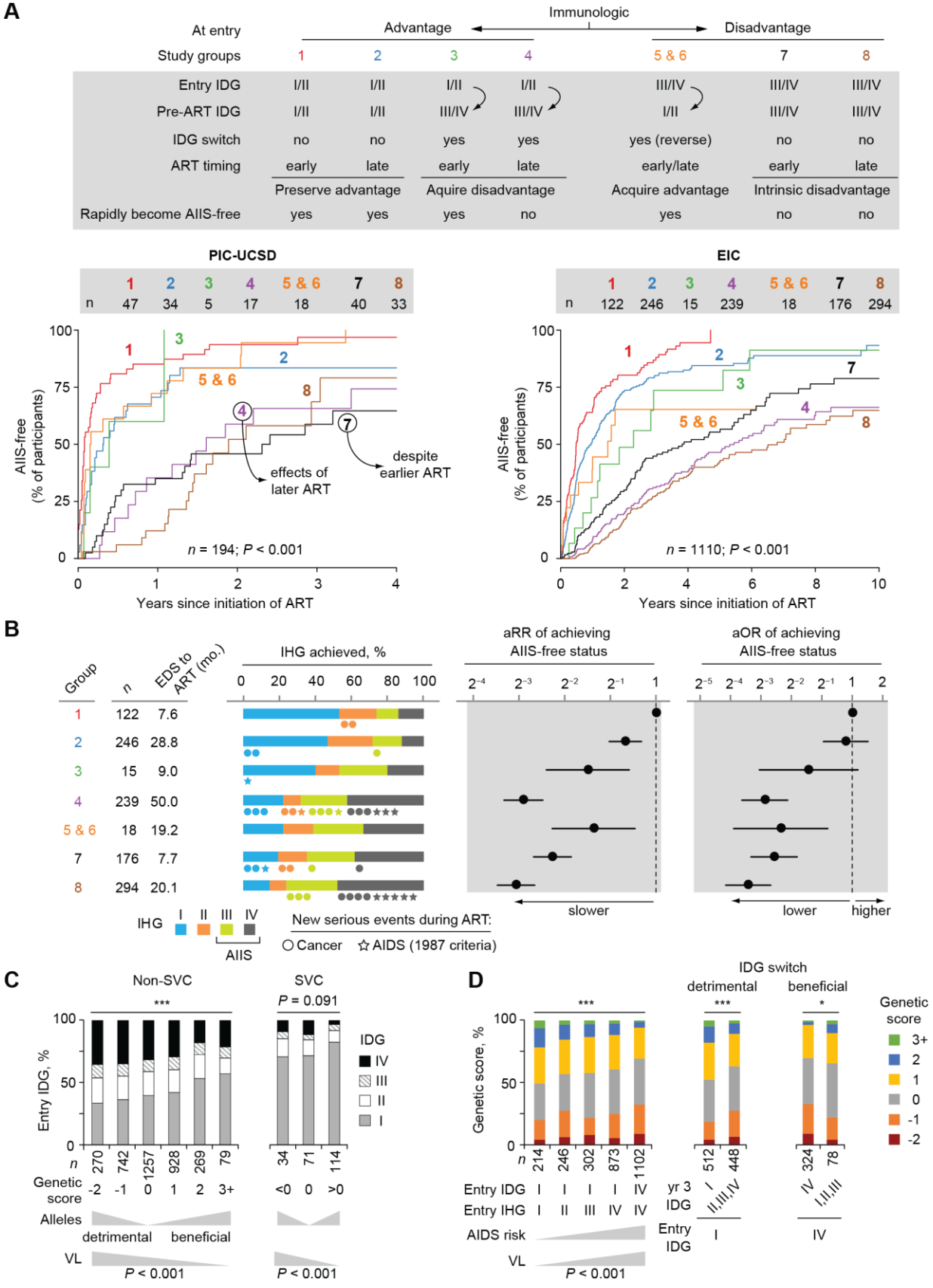

A
overall
earlier ART
later ART
P < 0.001
P = 0.015
IHG, %
n
Grp.
Age (yrs)
EIC
P < 0.001
P = 0.022
PIC-UCSD
Grp.
1 Entry
2 pre-ART
3 yrs 1-2
4 yrs 3-4
5 yrs 5-10
ART
IHG
IV
III
II
I
AIIS
B
HIV– low-risk (young)
HIV– low-risk (old)
HIV– FSW (young)
EIC earlier ART
>0 Behavioral activity score
PIC-UCSD overall
Pre-ART
IDG-I
IDG-II
IDG-III
IDG-IV
18 - 29
≥80
C
IDG-I
IDG-II
IDG-III
IDG-IV
Accumulated host antigenic burden pre-ART
low-risk younger (18-29yr)
low-risk older (≥80yr)
FSW with high behavioral scores
Post-ART host antigenic burden ranked according to HIV– populations
Reconstitution of AIIS-free status in HIV+

Figure 8. Immunologic disadvantage: causes and consequences during antiretroviral therapy (ART). (**A**) (Top) Characteristics of 8 study groups classified according to (i) immune damage grades (IDGs) at study entry (baseline) that associate with immunologic advantage vs. disadvantage, (ii) pre-ART IDG, and (iii) participants receiving ART earlier, defined as within 4 months from estimated date of infection (EDI) in the primary infection cohort-University of California San Diego (PIC-UCSD) and 12 months from estimated date of seroconversion (EDS) in the early infection cohort (EIC). (Bottom) Kaplan-Meier plots depicting rate ratios of achieving AIIS-free status in the indicated study groups of PIC-UCSD (left) and EIC (right) cohorts. *P*, log-rank test. **(B)** Proportion of best IHG achieved in EIC participants with corresponding adjusted rate ratios (aRR) and adjusted odds ratios (aOR) of achieving AIIS-free status during ART by same groups as in panel A. RR and OR are adjusted for calendar year of ART initiation, ART regimen, age, sex, and ethnicity. Depicted are the new serious AIDS and non-AIDS (cancer) adverse events that occurred in each group and by IHG achieved. **(C)** Distribution of entry IDG in EIC participants who are not spontaneous virologic controllers (non-SVC, left) and who are SVCs (right) by genetic score. *P*, χ^2 test for gene signature score distribution in non-SVC participants (top left), Fisher's exact test for prevalence of IDG-IV in SVCs by gene signature score (top right), and trend test for baseline HIV viral load (VL) by gene signature score. **(D)** Genetic score in EIC participants co-indexed by the indicated baseline IHGs and IDGs (left) and whether persons presenting with IDG-I or IDG-IV experienced a detrimental or beneficial IDG switch during therapy-naïve follow-up (right). Genetic score as defined in Table S16. *P*, χ^2 test (top) and linear regression (bottom for VL). **P*<0.05, ***P*<0.001.

Figure 8


A
Immunologic
At entry
Advantage
Disadvantage
Study groups
1 2 3 4 5 & 6 7 8
Entry IDG
Pre-ART IDG
IDG switch
ART timing
Preserve advantage
Aquire disadvantage
Acquire advantage
Intrinsic disadvantage
Rapidly become AIIS-free
PIC-UCSD
EIC
AIIS-free (% of participants)
Years since initiation of ART
n = 194; P < 0.001
n = 1110; P < 0.001
effects of later ART
despite earlier ART
B
Group
n
EDS to ART (mo.)
IHG achieved, %
aRR of achieving AIIS-free status
aOR of achieving AIIS-free status
slower
lower
higher
IHG
AIIS
New serious events during ART:
Cancer
AIDS (1987 criteria)
C
Non-SVC
SVC
P = 0.091
Entry IDG, %
IDG
Genetic score
Alleles
detrimental
beneficial
VL
P < 0.001
D
IDG switch
detrimental
beneficial
Genetic score, %
Genetic score
Entry IDG
Entry IHG
AIDS risk
yr 3 IDG
Entry IDG
P < 0.001