Open Forum Infectious Diseases

MAJOR ARTICLE

Monitoring Circulating Immune Checkpoint Proteins as Predictors of Non-AIDS Morbid Events in People With HIV Initiating Antiretroviral Therapy

Thomas A.Premeaux,[1] Carlee B.Moser,[2] AshleyMcKhann,[2] MartinHoenigl,[3] Stephen T.Yeung,[1] Alina P. S.Pang,[1] Michael J.Corley,[1] Michael M.Lederman,[4] Alan L.Landay,[5] SaraGianella,[3] and Lishomwa C.Ndhlovu[1]; for the Adult Clinical Trials Group NWCS 411 study team

[1]Division of Infectious Diseases, Department of Medicine, Weill Cornell Medicine, New York, New York, USA, [2]Center for Biostatistics in AIDS Research in the Department of Biostatistics, Harvard T.H. Chan School of Public Health, Boston, Massachusetts, USA, [3]Division of Infectious Diseases, Department of Medicine, University of California San Diego, San Diego, California, USA, [4]Division of Infectious Diseases and HIV Medicine, Department of Medicine, Case Western Reserve University, Cleveland, Ohio, USA, and [5]Department of Internal Medicine, Rush University Medical Center, Chicago, Illinois, USA

Background. Although cell surface immune checkpoint proteins (ICPs) such as PD-1 expressed on T cells are associated with T-cell exhaustion, HIV disease progression, and AIDS events, they have shown limited utility in predicting non-AIDS morbidity. Given that ICPs also exist in soluble forms and are elevated in ART-treated HIV infection, we tested the hypothesis that soluble ICPs may be predictive of non-AIDS events in adults initiating ART.

Methods. Utilizing a nested case–control study from the AIDS Clinical Trials Group ALLRT cohort, we measured plasma levels of 15 soluble inhibitory and activating ICPs by Luminex. Participants (134 cases, 292 matched controls) were evaluated pre-ART, a year post-ART, and immediately preceding a non-AIDS event, which included myocardial infarction (MI)/stroke, malignancy, serious bacterial infection, and nonaccidental death.

Results. Conditional logistic regression analysis determined that higher levels of soluble CD27 were associated with increased risk of non-AIDS events at all time points. Higher levels of CD40 at baseline and pre-event and CD80 at pre-event were associated with increased risk of non-AIDS events. Examining specific non-AIDS events, multiple ICPs were associated with malignancy at baseline and pre-event, whereas only higher CD27 levels were associated with increased risk of MI/stroke at year 1 and pre-event.

Conclusions. While select soluble ICPs were associated with non-AIDS events, CD27 emerged as a consistent marker irrespective of ART. Our data may offer guidance on new targets for early clinical monitoring in people with HIV who remain at greater risk of specific non-AIDS events.

Keywords. antiretroviral therapy; biomarkers; HIV; immune checkpoints; morbidity.

Despite the success of effective antiretroviral therapy (ART), people with HIV (PWH) disproportionately suffer from comorbidities, such as cardiovascular disease and non-AIDS-associated malignancies, compared with the general population [1]. Compromised immune function, chronic activation of immune cells, and inflammation remain hallmarks of ART-treated HIV infection, and these factors are often thought to drive the occurrence of non-AIDS morbidities [2, 3]. Multiple mechanisms likely contribute to the increased morbidity risk and immune dysfunction in PWH, including but not limited to the direct effects of HIV persistence, gut microbial translocation, increased circulation of inflammatory lipids, and co-infection persistence [1]. While various biomarkers associated with inflammation, immune activation, and microbial translocation have been linked to non-AIDS events [4, 5], the immune regulatory pathways involved in the pathogenesis of these complications are not fully understood. Further elucidating these associated pathways could facilitate the development of ideal comprehensive clinical trial end points and the discovery of predictors for use in the clinical management of PWH on ART.

The persistent activation, exhaustion, and senescence of $CD4^+$ and $CD8^+$ T [6], natural killer (NK) [7], and B cells [8] have been associated with HIV immunopathogenesis and AIDS-related complications; however, few studies have examined whether lymphocyte dysfunction characteristics are informative for non-AIDS morbid outcomes in PWH on ART. The co-activation and dysfunction of T, B, and NK lymphocytes can be regulated by several immune checkpoint co-stimulatory and

Received 4 October 2021; editorial decision 4 November 2021; accepted 10 January 2022; published online 21 January 2022.

Correspondence: Lishomwa C. Ndhlovu, MD, PhD, Division of Infectious Diseases, Department of Medicine, Weill Cornell Medicine, 413 East 69th St., BRB, New York, NY 10021 (lndhlovu@med.cornell.edu).

Open Forum Infectious Diseases®2021

https://doi.org/10.1093/ofid/ofab570

inhibitory receptor/ligand interactions, and the overexpression of these proteins, such as programmed cell death protein 1 (PD-1) and T-cell immunoglobulin and mucin-domain containing 3 (TIM-3), has been shown to contribute to lymphocyte dysfunction and viral persistence in HIV [9]. Immune checkpoint proteins (ICPs) also exist in soluble forms at normal physiological conditions, originating from the alternative splicing of mRNA or through the protease-mediated cleavage of their membrane-bound form [10]. There is ongoing and increasing interest in measuring soluble ICPs as many have been elevated in the plasma of individuals with cancer, HIV, or other inflammatory diseases, suggesting that they could serve as promising predictive biomarkers and may be involved in disease pathology [11–13]. Our group and others have identified soluble ICPs, such as TIM-3 and CD27, associated with HIV disease progression and immune activation [14, 15], yet the utility of ICPs as early biomarkers of non-AIDS events remains unclear.

Given the increased incidence of cardiovascular events and cancer in PWH and the established link between ICPs and these comorbid outcomes in the general population, we aimed to measure and identify soluble ICPs in plasma as biomarkers of the onset of non-AIDS events. To investigate this relationship, plasma levels of soluble co-stimulatory and inhibitory checkpoint proteins were measured before and after ART initiation in PWH who did or did not experience a non-AIDS event. We tested the predictive efficacy of these ICPs for total non-AIDS events and specific events, including mortality, cardiovascular events (myocardial infarction [MI] or stroke), and non-AIDS-defining malignancy. Ultimately, further understanding the link between these novel biomarkers of persistent lymphocyte activation and dysfunction and comorbid outcomes in HIV could uncover potential targets for therapeutic intervention and improve the clinical monitoring of PWH who are virologically suppressed on ART.

METHODS

Study Population

NWCS 411 is an ALLRT-nested case–control study of PWH enrolled from 2001 to 2009, which examined potential predictive biomarkers and their relationships with non-AIDS events and death. This study builds off a previous case–control study, which examined a multitude of inflammatory and immune activation markers and found associations between several markers of immune activation and non-AIDS events and death, as previously described [4]. Cases were defined as participants who experienced an MI or a stroke, a non-AIDS-defining malignancy, serious bacterial infection, or who died from a nonaccidental non-AIDS-related event. For each case, 1–3 participants (controls) with an event-free follow-up equal to or greater than the relevant case and matched for age and sex at the time of the visit, pre-ART $CD4^+$ T-cell count, and ART regimen at year 1 were evaluated.

All participants were ART-naive when enrolled in their original ACTG study and had plasma HIV RNA <400 copies/mL at 1 year post–ART initiation. Most study participants maintained their plasma HIV RNA <400 copies/mL after year 1; participants with values >400 copies/mL were included if preceding and subsequent HIV RNA values were <400 copies/mL without a change in ART regimen. For the events, the data were reported by sites following standardized ACTG diagnosis criteria. Cancer, MI, and serious bacterial infection events in the ALLRT analysis data sets were reviewed and confirmed by the ALLRT team. The nonaccidental death designation was based on thorough review of reported cause of death by statisticians and study chairs. The team (ALLRT/NWCS) did not have direct access to medical records or death registries.

Soluble Immune Checkpoint Quantification

Stored plasma samples from the time before ART initiation (pre-ART), 1 year (48–64 weeks) after ART initiation, and the time (visit) proximal to/immediately preceding the non-AIDS event (and corresponding time point in controls) were tested for the following soluble co-stimulatory and inhibitory proteins using a multiplex immunoassay (Milliplex): cluster of differentiation 27 (CD27), CD28, CD40, CD80 (B7-1), CD86 (B7-2), glucocorticoid-induced TNFR-related protein (GITR), GITR ligand (GITRL), herpesvirus entry mediator (HVEM), B- and T-lymphocyte attenuator (BTLA), inducible T-cell co-stimulator (ICOS), cytotoxin T-lymphocyte-associated protein 4 (CTLA-4), lymphocyte-activation gene 3 (LAG-3), programmed cell death protein 1 (PD-1), programmed death-ligand 1 (PD-L1), and T-cell immunoglobulin and mucin-domain containing 3 (TIM-3). Data were acquired on a Luminex 200 analyzer (Luminex) and analyzed using MILLIPLEX Analyst software (Millipore). All samples were analyzed in duplicate.

Statistics

Demographic and clinical characteristics are presented using the median (interquartile range [IQR]) for continuous variables and frequency for categorical variables. Soluble markers were $\log_{10}$-transformed before all analyses. Spearman rank correlations were used to assess the associations among ICPs, with pertinent clinical assessments and previously assessed biomarkers at each time point among the controls. Differences in paired data from baseline to year 1 were summarized with Hodges-Lehmann estimates and 95% CIs. Conditional logistic regression analysis assessed associations of soluble ICPs with non-AIDS events. Models were adjusted for pertinent covariates at each time point, including the following potential confounders: HIV disease measures (concurrent $\log_{10}$ HIV RNA levels at baseline, $CD4^+$ T-cell counts at year 1 and pre-event), chronic hepatitis B or C, intravenous drug use, waist-to-hip ratio, clinician-diagnosed diabetes, clinician-diagnosed hypertension, use of antihypertensive or lipid-lowering agents,

smoking status, and family history of MI. Additional models considered associations with adjustment for biomarkers previously linked to non-AIDS events. All analyses were performed in SAS, version 9.4 (SAS Institute).

RESULTS

Description of Study Population

For our analysis, we included 3 time points: baseline (pre-ART; 66 cases, 97 controls), 1 year post-ART (112 cases, 211 controls), and immediately preceding an event (89 cases, 162 controls). Case and control groups were comparable in age and sex at time of visit, $CD4^+$ T-cell counts at baseline (pre-ART) and ART regimen at week 48 (whether it contained a protease inhibitor or abacavir), and parent ACTG study (Table 1). At baseline, participants were predominately male (84%), with a median (IQR) age of 45 (39–51) years, 213 (79–334) cells/μL $CD4^+$ T-cell count, and 4.8 (4.4–5.4) $\log_{10}$ copies/mL plasma HIV RNA. Median (IQR) $CD4^+$ T-cell counts at year 1 were 404 (269–561) cells/μL for controls and 347 (229–479) cells/μL for cases. Among cases, non-AIDS events occurred at a median (IQR) of 2.8 (1.7–4.6) years after ART initiation and 10.5 (6–19) weeks from the pre-event time point and included nonaccidental deaths (13.4%), MI/stroke (28.4%), malignancy (37.3%), and serious bacterial infection (26.9%).

Co-stimulatory/Inhibitory Immune Checkpoint Protein Distributions and Correlates

Distributions of soluble co-stimulatory and inhibitory ICPs at each time point for cases and controls are illustrated in Figure 1. Of note, ICP levels of CD27 and TIM-3 decreased from baseline to 1 year after ART initiation in both cases and controls, while CD28, BTLA, and CD80 increased (Supplementary Table 1). We next assessed potential associations among ICPs and correlations with biomarkers of inflammation, immune activation, coagulopathy, and microbial translocation as well as pre-ART factors. ICPs were highly associated with one another at all time points ($r \geq 0.60$) (Supplementary Figure 1A), apart from weak correlations with CD27, HVEM, LAG-3, and TIM-3. Overall,

Table 1. Cohort Demographic and Clinical Characteristics at Baseline

Characteristic	Case (n = 134)	Control (n = 292)	Total (n = 426)
Age at parent study entry, y	47 (40–53)	44 (39–50)	45 (39–51)
Regimens evaluated, by parent study			
ACTG 384:(AZT + 3TC vs d4T + ddI) + (EFV vs NFV vs NFV + EFV)/ ACTG 388: (AZT + 3TC vs d4T + 3TC) + (IDV vs NFV vs IDV + NFV)	40 (30)	85 (29)	125 (29)
A5014: NVP + [LPV/r vs (ABC + 3TC + d4T)]/ A5095: AZT/3TC + (ABC vs EFV vs ABC + EFV)/ A5142: (EFV + AZT/d4T + 3TC) vs (LPV/r + AZT/d4T + 3TC) vs (EFV + LPV/r)	62 (46)	144 (49)	206 (48)
A5202: (ABC/3TC vs TFV/FTC) + (ATV/r vs EFV)	32 (24)	63 (22)	95 (22)
Sex			
Male	112 (84)	247 (85)	359 (84)
Female	22 (16)	45 (15)	67 (16)
Race/ethnicity			
White non-Hispanic	70 (52	138 (47)	208 (49)
Black non-Hispanic	48 (36)	82 (28)	130 (31)
Hispanic (regardless of race)	15 (11)	61 (21)	76 (18)
Other	1 (1)	11 (4)	12 (3)
Baseline $CD4^+$ T cell count, cells/μL	207 (87–334)	220 (76–332)	213 (79–334)
Baseline $\log_{10}$ HIV-1 RNA, copies/mL	4.8 (4.4–5.3)	4.8 (4.4–5.4)	4.8 (4.4–5.4)
Chronic hepatitis B/C status	33 (25)	29 (10)	62 (15)
Current or previous injection drug use	17 (13)	26 (9)	43 (10)
Waist-to-hip ratio	0.92 (0.89–0.96)	0.92 (0.88–0.97)	0.92 (0.89–0.97)
History of clinician-diagnosed diabetes	11 (8)	15 (5)	26 (6)
History of hypertension	43 (32)	55 (19)	98 (23)
Use of antihypertensive or lipid-lowering agents	30 (22)	40 (14)	70 (16)
Current or past smoker	100 (75)	159 (54)	259 (61)
Family history of myocardial infarction	28 (21)	43 (15)	71 (17)

Categorical variables are represented as frequency (%), and continuous variables as median (interquartile range).

Abbreviations: 3TC, lamivudine; ABC, abacavir; ATZ/r, ritonavir-boosted atazanavir; AZT, zidovdine; d4T, stavudine; ddI, didanosine; EFV, efavirenz; FTC, emtricitabine; IDV, indinavir; LPV/r, ritonavir-boosted lopinavir; NFV, nelfinavir; NVP, nevirapine; TFV, tenofovir.

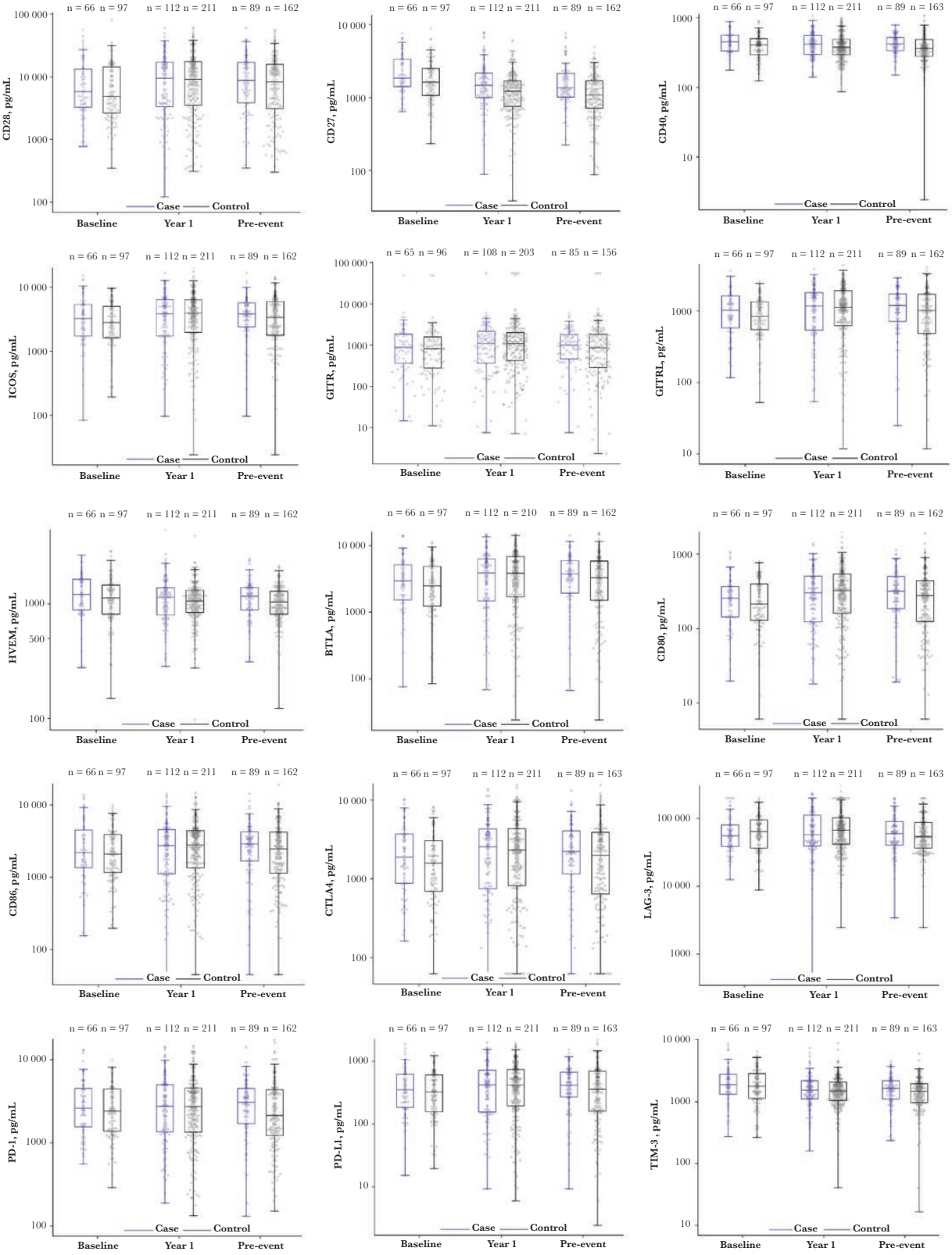

Figure 1. Distribution of soluble co-stimulatory and inhibitory checkpoints. Levels among cases (blue) and controls (black) at pre–ART initiation (baseline), a year after ART initiation (year 1), and visit immediately preceding a non-AIDS event (pre-event). Jitter plots including median and interquartile range are displayed. Abbreviation: ART, antiretroviral therapy.

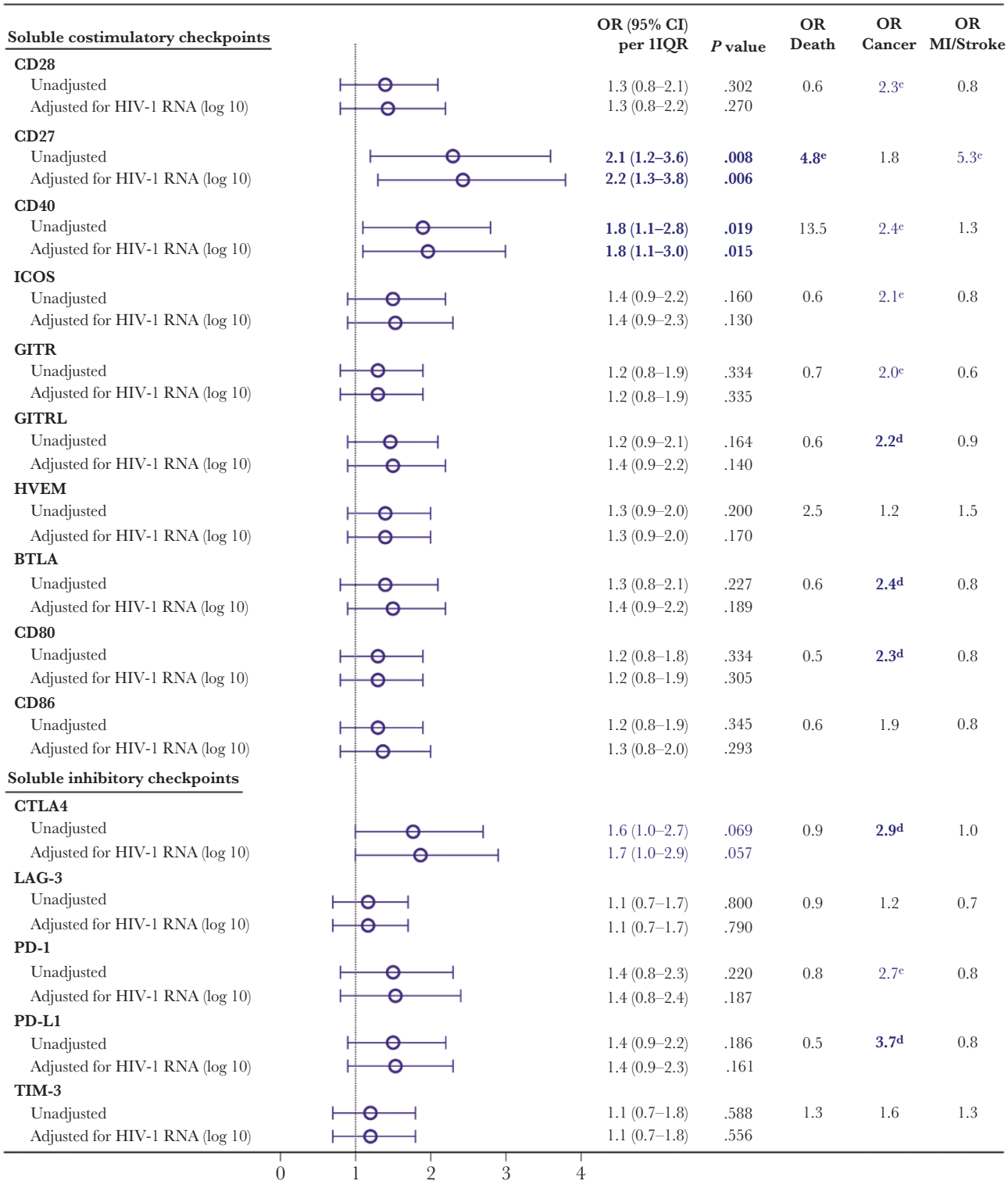

Figure 2. Soluble co-stimulatory and inhibitory checkpoint protein levels and odds ratios of having a non-AIDS event at baseline (pre-ART). Adjusted analyses controlled for concurrent HIV viral load. [d]$P < .05$; [e]$P < .1 > .05$. Abbreviations: ART, antiretroviral therapy; MI, myocardial infarction; OR, odds ratio.

ICPs did not correlate with previous biomarkers associated with non-AIDS events (Supplementary Figure 1B), except for HVEM, TIM-3, and CD27. HVEM and TIM-3 correlated with suPAR and TNFRI/II at baseline and pre-event ($r \geq 0.33$) and TNFRI/II at year 1 ($r \geq 0.38$); CD27 correlated with TNFRII at baseline ($r = 0.39$). Among pre-ART factors, baseline for the ICPs HVEM and TIM-3 inversely correlated with $CD4^+$ T-cell count ($r < -0.22$) (Supplementary Figure 1C), TIM-3 correlated

with HIV RNA level ($r = 0.36$), and CD27, CD40, HVEM, and TIM-3 correlated with age ($r \geq 0.22$).

Pre-ART Soluble Immune Checkpoint Proteins Levels and Non-AIDS Events

Higher baseline plasma levels of CD27 (odds ratio [OR] per 1 IQR, 2.1; 95% CI, 1.2–3.6; $P = .008$) and CD40 (OR, 1.8; 95% CI, 1.1–2.8; $P = .019$) were associated with increased risk of non-AIDS events in unadjusted conditional logistic regression analysis (Figure 2); however, associations were not observed with other ICPs at baseline. CD27 and CD40 associations with non-AIDS events remained significant after adjustment for HIV RNA levels and other potential confounders (Supplementary Tables 2–3), except for when adjusted for waist-to-hip ratio. When adjusted for biomarkers previously shown to be predictive of non-AIDS events, associations were slightly attenuated for CD27 when adjusted for suPAR, and CD40 with adjustments for suPAR, EBV DNA, and CMV DNA (Supplementary Tables 4 and 5). Examining specific non-AIDS events, higher levels of CD27 were associated with increased risks of death (n = 9 events; OR, 4.8; 95% CI, 0.8–28.1) and MI/stroke (n = 15 events; OR, 5.3; 95% CI, 0.8–35.4), and higher levels of CD40 were associated with increased risk of malignancy (n = 28 events; OR, 2.4; 95% CI, 0.9–6.9). Other IPCs were associated with increased risk of specific non-AIDS events and included BTLA, GITRL, CD80, CTLA4, and PD-L1, which associated with increased risk of malignancy (OR, 2.2–3.7).

Year 1 Soluble Immune Checkpoint Proteins Levels and Non-AIDS Events

Conditional logistic regression results are detailed in Figure 3. Higher levels of CD27 were associated with increased risk of non-AIDS events at year 1 (OR, 1.6; 95% CI, 1.2–2.2; $P = .001$), while no significant associations were found with other soluble co-stimulatory proteins. This association with CD27 remained after adjustments for $CD4^+$ T-cell counts, other pertinent confounders, and biomarkers previously shown to be predictive of non-AIDS events (Supplementary Table 6–7). Inhibitory checkpoint marker levels at year 1 were also not associated with increased risk of having a non-AIDS event. Regarding specific non-AIDS events, higher levels of CD27 and TIM-3 were associated with increased risk of MI/stroke (n = 32 events; OR, 2.3–2.9).

Pre-event Soluble Immune Checkpoint Protein Levels and Non-AIDS Events

Figure 4 illustrates the associations between pre-event levels of soluble co-stimulatory proteins and non-AIDS events. Higher pre-event levels of CD27 (OR, 2.1; 95% CI, 1.4–3.3; $P < .001$), CD40 (OR, 1.7; 95% CI, 1.2–2.5; $P = .008$), and CD80 (OR, 1.6; 95% CI, 1.1–2.4; $P = .017$) were associated with increased risk of non-AIDS events. CD27 associations remained after adjustments for $CD4^+$ T-cell counts, confounders, and other biomarkers, while CD40 and CD80 associations were attenuated when adjusting for hep B/C, smoking, and IL-6 (Supplementary Tables 8–13). The only inhibitory checkpoint marker associated with non-AIDS events was PD-1 (OR, 1.5; 95% CI, 1.0–2.2; $P = .045$); however, this association was attenuated when adjusting for $CD4^+$ T-cell count. Examining specific non-AIDS events, higher CD27 associated with an increased risk of MI/stroke (n = 23 events; OR, 4.1; 95% CI, 1.3–12.8), while CD40, CD80, GITRL, LAG-3, PD-1, and PD-L1 were all associated with increased risk of malignancy (n = 35 events; OR, 2.1–4.1).

DISCUSSION

Lymphocyte immune perturbations can be mediated through the immune checkpoint axis, many belonging to the CD28 and TNFR superfamilies [16, 17]. While phenotypic analysis expression of these checkpoints on specific cellular subsets has been extensively studied in HIV, these ICPs also exist in soluble forms and may give a proxy of overall lymphocyte activation and exhaustion status. Here we report that of 15 soluble ICPs, plasma CD27, CD40, and CD80 were associated with non-AIDS events overall. We demonstrate that many ICPs, particularly GITRL, CD80, and PD-L1, were associated with malignancy before and after ART initiation. However, soluble CD28, ICOS, HVEM, and CD86 showed no association with total or specific non-AIDS events. These findings reveal a previously unappreciated role for soluble ICPs in PWH that may assist in predicting non-AIDS events.

A major outcome of this study was identifying circulating levels of CD27 as a predictor of non-AIDS event outcomes throughout the course of untreated and treated chronic HIV infection. Cell surface CD27 is expressed on T cells and activated B cells and through binding to the ligand CD70 and provides co-stimulatory signals to enhance proliferation and increase effector function [18]. However, tonic CD27-CD70 interaction is thought to cause immune dysregulation [19]. In PWH, CD27 on effector memory $CD8^+$ CTLs promotes their long-term survival, while an upregulation on $CD4^+$ T cells is associated with poor proliferative responses [20, 21]. Soluble CD27 can be derived from shedding by activated lymphocytes [22]. Elevated plasma levels of CD27 are associated with HIV disease progression and have been suggested as a surrogate marker of immune activation during HIV infection [15, 23, 24]. Higher pre-ART levels of CD27 have also been associated with AIDS-defining malignancies and mortality that occurred after the initiation of suppressive treatment [25, 26]. Further understanding CD27's significance throughout the course of HIV would reveal its potential as a clinical indicator for total comorbid complications during durable and suppressive ART.

To our knowledge, the soluble forms of CD40 and CD80 have not previously been associated with HIV severe outcomes. Cell-bound CD40 is mainly regulated to antigen-presenting cells (APCs) and upon binding to its respective ligand, CD40L, induces their activation. CD80 is expressed on activated APCs,

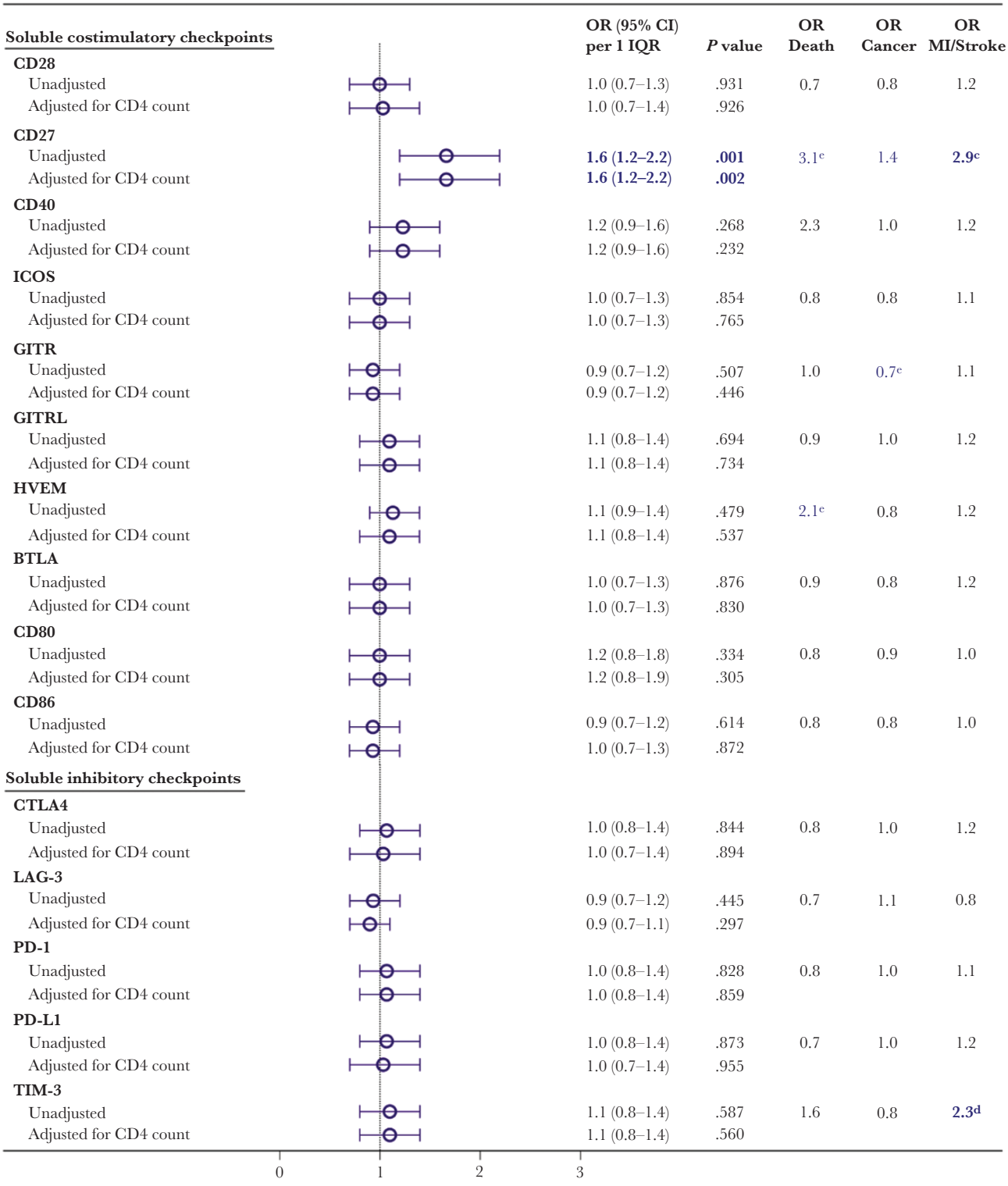

Figure 3. Soluble co-stimulatory and inhibitory checkpoint protein levels and odds ratios of having a non-AIDS event at year 1. Adjusted analyses controlled for concurrent CD4 count. [c]$P < .01$; [d]$P < .05$; [e]$P < .1 > .05$. Abbreviations: MI, myocardial infarction; OR, odds ratio.

monocytes, and B cells and binds to CD28 and CTLA-4 to activate or inhibit T-cell responses, respectively [17]. Levels of plasma CD40, as well as CD27, were higher in individuals before death due to alcoholic hepatitis compared with those that survived, with CD40 being able to predict a 90-day mortality risk [12]. While not observed in our study, CD40 in blood has been associated with organ damage, particularly stroke, in those with hypertension outside the context of HIV infection [27].

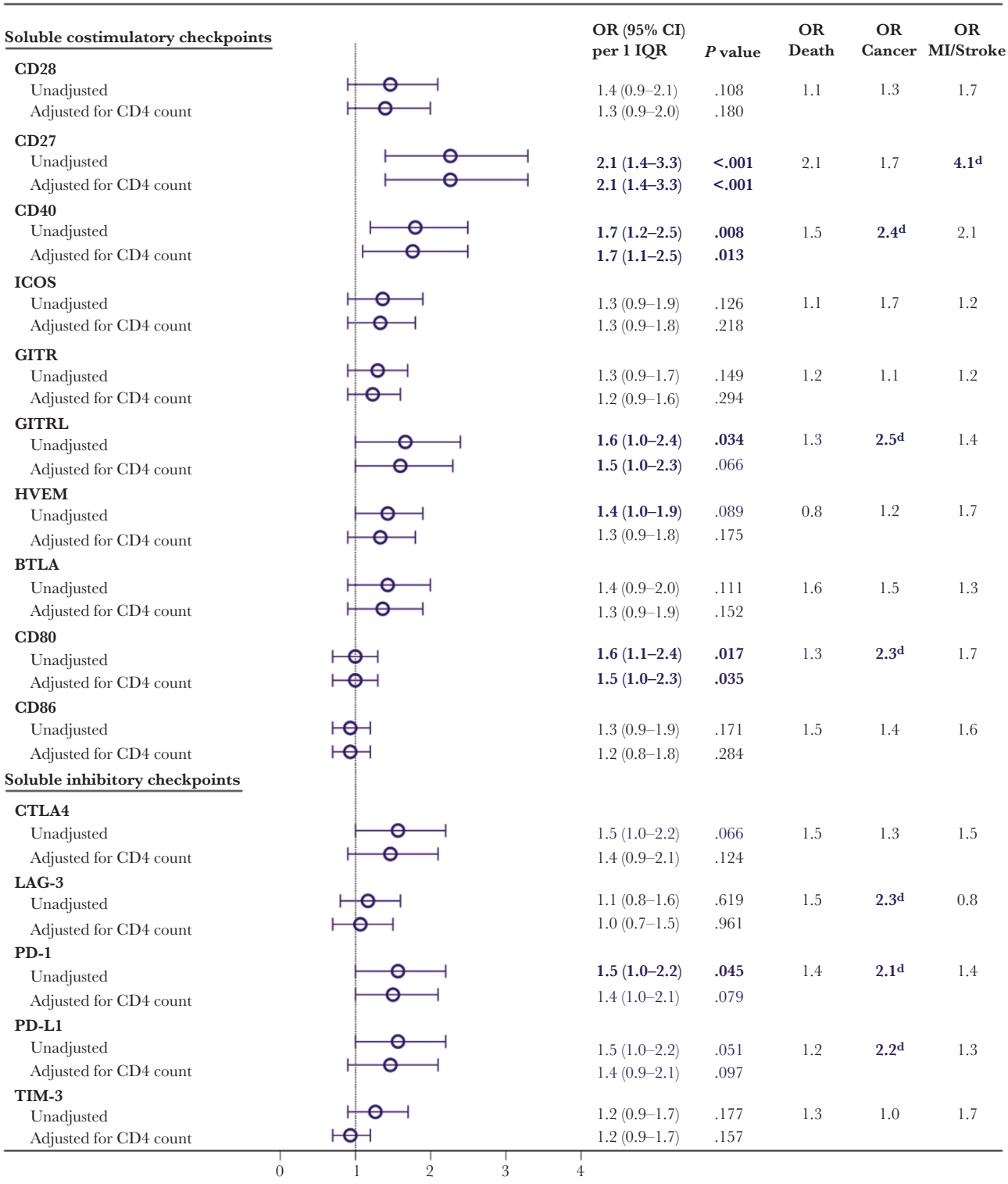

Figure 4. Soluble co-stimulatory and inhibitory checkpoint protein levels and odds ratios of having a non-AIDS event at pre-event. Adjusted analyses controlled for concurrent CD4 count. [d]$P < .05$. Abbreviations: MI, myocardial infarction; OR, odds ratio.

Interestingly, in our study most soluble ICPs did not correlate with biomarkers associated with inflammatory, myeloid activation, and microbial translocation mechanisms, indicating that these are possibly independent pathways linked to non-AIDS events and revealing an underappreciated role for these markers in the context of HIV infection. ICPs were also highly intercorrelated, illustrating that these additional immune activation and exhaustion pathways could be intertwined with one another. Many

of the inhibitory immune checkpoints become expressed after activation to TCR and BCR mechanisms. Whether levels we find in circulation represent the activation or exhaustive state of peripheral or tissue-resident lymphocytes should be determined.

Another important finding of our study is that we identified many soluble co-stimulatory and inhibitory ICPs associated specifically with the onset of cancer, particularly at pre-ART and pre-event time points. ICPs have been heavily investigated in cancer as potential prognostic markers. Plasma CD40 was previously identified as a potential prognostic marker of malignant pleural effusion with non–small cell lung cancer [28]. Elevated levels of CD80 have been shown to be associated with the incidence of various hematologic malignancies with malignant cells, in addition to APCs, which have been suggested as potential sources [29]. Additionally, soluble forms of PD-1, PD-L1, and LAG-3 are thought to have predictive and prognostic significance in various cancers as well as directly correlating with antitumor immunity [30, 31].

ICPs have been shown to be functional in their soluble forms in vitro by acting as antagonist decoys that competitively regulate their membrane-bound counterparts. For instance, CD27 cleaved from T cells has been shown to bind membrane-associated CD70 on APCs, facilitating competition between T cells for access to MHC:peptide complexes [32], while soluble CD40 can bind membranous CD154 and inhibit B-cell activity [33, 34]. Soluble ICPs can also directly stimulate activation and/or inhibition checkpoint pathways. Soluble CD27 has been shown to directly contribute to the activation of T cells and B cells, leading to a state of hyperactivation [22]. Soluble CD80, on the other hand, can enhance antitumor responses by promoting tumor-infiltrating lymphocytes by simultaneously inhibiting PD-1 and providing co-stimulation of antitumor T-cell activity [35]. Immunotherapeutic strategies targeting ICP pathways are ongoing in clinical trials for cancer and are being investigated as HIV curative approaches by enhancing anti-HIV T-cell responses [36, 37]. Whether fluctuating levels of the soluble forms of ICPs play a role in altering the efficacy of these strategies targeting membrane-bound immune checkpoint receptors or ligands should be determined.

Our study has several limitations. Case and control numbers varied across time points due to limitations on specimen availability, which limits the ability to evaluate longitudinal changes and could explain some of our inconsistent findings. ICPs were found to be highly correlated with one another. However, these intercorrelations could be driven by the multiplex due to a systemic artifact that renders these markers close together. Further studies will need to determine if these associations might represent the debris that remains in circulation after cellular turnover or death. Our analysis only consisted of adjustments for single variables. This was to check the consistency of the results when considering potential confounders rather than to create a predictive model. Of note, the prevalence of hep B/C was rather dissimilar between cases and controls. Although we adjusted for hep B/C in our analyses, the differences between the 2 groups could still potentially influence their relative ICP expression. Many study participants were on ART regimens that have been phased out and are no longer recommended for initial treatment of HIV infection, including D4T, which can cause serious side effects such as peripheral neuropathy and lipoatrophy [38]. However, only 55 (17%) of participants were on D4T at year 1, and case and controls were matched by ART regimen. Furthermore, as current first-line regimens, such as integrase strand transfer inhibitors, were not represented in the cohort, it would be essential to determine whether these findings could be extrapolated to a contemporary PWH population who now start modern ART much sooner after HIV diagnosis and achieve virologic suppression at a faster rate. Finally, HIV participants within our cohort had relatively lower $CD4^+$ T-cell counts as compared with those who receive modern ART regimens immediately upon diagnosis, and women were under-represented, of importance as there are sex-related differences in the risk of select non-AIDS comorbidities [39].

While our findings of soluble ICPs as potential predictors for severe outcomes are compelling, the next logical step would be to confirm that these associations are consistent using an HIV cohort with a modern and early initiated ART regimen. Also, larger prospective studies evaluating a broader assessment of non-AIDS events will allow the confirmation of these markers at various stages of disease and ART exposure. Of practical interest, their potential use as surrogate markers for end points in clinical trials targeting inflammation and immune dysfunction aimed to alleviate morbidity and mortality in PWH on ART should be evaluated. These studies should focus on whether individual ICPs or their inclusion in composite panels can serve as optimal predictors and monitoring tools. Furthermore, subsequent studies should determine if there is a causative link between soluble ICPs, perturbations in lymphocyte function, and comorbid events, which may facilitate uncovering the molecular mechanisms soluble ICPs may be involved in during untreated and treated HIV infection and lay groundwork for potential therapeutic targets.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Acknowledgments

We would like to thank all study participants and clinical staff involved in the ACTG ALLRT study and the James B. Pendleton Charitable Trust.

Financial support. The research reported in this publication was supported by National Institute of Health grants UM1AI068634, UM1AI106701, UM1AI068636 (M.M.L.), R01MH112457 (L.C.N.), R01AI147821 (S.G.), DA051915 (S.G.), and UM1AI068634 (C.B.M., A.M.).

Disclaimer. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

Potential conflicts of interest. L.C.N. has served on an advisory board for AbbVie, ViiV, and Cytodyn for work unrelated to this project. M.H. has received project funding from Gilead, Pfizer, and Astellas. M.M.L. has consulted for Lilly and has received competitive grant funding from Gilead for unrelated work. All other authors declare no competing interests. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

Author contributions. T.A.P. and L.C.N. wrote the manuscript; T.A.P. conducted soluble marker acquisition and data interpretation; C.B.M. and A.M. contributed to data analysis and interpretation; S.T.Y., A.P.S.P., M.J.L., M.H., E.I.L., M.M.L., and A.L.L. provided critical review of the manuscript. L.C.N., S.G., and C.B.M. contributed to study design and concept. All authors reviewed the manuscript.

Patient consent. Participants (or, for minors, their parent or legal guardian) provided written informed consent, and institutional review board approval for ALLRT was obtained by each ACTG site.

Availability of data and materials. Individual participant data and a data dictionary defining each field in the set will be made available to investigators on a case-by-case basis via request to the AIDS Clinical Trials Group (ACTG) via the following link: https://submit.mis.s-3.net. Completion of an ACTG Data Use Agreement may be required.

References

1. Deeks SG, Lewin SR, Havlir DV. The end of AIDS: HIV infection as a chronic disease. Lancet **2013**; 382:1525–33.
2. Deeks SG, Tracy R, Douek DC. Systemic effects of inflammation on health during chronic HIV infection. Immunity **2013**; 39:633–45.
3. Schouten J, Wit FW, Stolte IG, et al; AGEhIV Cohort Study Group. Cross-sectional comparison of the prevalence of age-associated comorbidities and their risk factors between HIV-infected and uninfected individuals: the AGEhIV cohort study. Clin Infect Dis **2014**; 59:1787–97.
4. Tenorio AR, Zheng Y, Bosch RJ, et al. Soluble markers of inflammation and coagulation but not T-cell activation predict non-AIDS-defining morbid events during suppressive antiretroviral treatment. J Infect Dis **2014**; 210:1248–59.
5. Hoenigl M, Moser CB, Funderburg N, et al; Adult Clinical Trials Group NWCS 411 study team. Soluble urokinase plasminogen activator receptor is predictive of non-AIDS events during antiretroviral therapy-mediated viral suppression. Clin Infect Dis **2019**; 69:676–86.
6. Fenwick C, Joo V, Jacquier P, et al. T-cell exhaustion in HIV infection. Immunol Rev **2019**; 292:149–63.
7. Costanzo MC, Kim D, Creegan M, et al. Transcriptomic signatures of NK cells suggest impaired responsiveness in HIV-1 infection and increased activity post-vaccination. Nat Commun **2018**; 9:1212.
8. De Milito A, Nilsson A, Titanji K, et al. Mechanisms of hypergammaglobulinemia and impaired antigen-specific humoral immunity in HIV-1 infection. Blood **2004**; 103:2180–6.
9. Fromentin R, Bakeman W, Lawani MB, et al. CD4+ T cells expressing PD-1, TIGIT and LAG-3 contribute to HIV persistence during ART. PLoS Pathog **2016**; 12:e1005761.
10. Nielsen C, Ohm-Laursen L, Barington T, et al. Alternative splice variants of the human PD-1 gene. Cell Immunol **2005**; 235:109–16.
11. Gu D, Ao X, Yang Y, et al. Soluble immune checkpoints in cancer: production, function and biological significance. J Immunother Cancer **2018**; 6:132.
12. Li W, Xia Y, Yang J, et al. Immune checkpoint axes are dysregulated in patients with alcoholic hepatitis. Hepatol Commun **2020**; 4:588–605.
13. Riva A, Chokshi S. Immune checkpoint receptors: homeostatic regulators of immunity. Hepatol Int **2018**; 12:223–36.
14. Clayton KL, Douglas-Vail MB, Nur-ur Rahman AK, et al. Soluble T cell immunoglobulin mucin domain 3 is shed from CD8+ T cells by the sheddase ADAM10, is increased in plasma during untreated HIV infection, and correlates with HIV disease progression. J Virol **2015**; 89:3723–36.
15. De Milito A, Aleman S, Marenzi R, et al. Plasma levels of soluble CD27: a simple marker to monitor immune activation during potent antiretroviral therapy in HIV-1-infected subjects. Clin Exp Immunol **2002**; 127:486–94.
16. Aggarwal BB. Signalling pathways of the TNF superfamily: a double-edged sword. Nat Rev Immunol **2003**; 3:745–56.
17. Sharpe AH, Freeman GJ. The B7-CD28 superfamily. Nat Rev Immunol **2002**; 2:116–26.
18. Hintzen RQ, Lens SM, Lammers K, et al. Engagement of CD27 with its ligand CD70 provides a second signal for T cell activation. J Immunol **1995**; 154:2612–23.
19. Nolte MA, van Olffen RW, van Gisbergen KP, van Lier RA. Timing and tuning of CD27-CD70 interactions: the impact of signal strength in setting the balance between adaptive responses and immunopathology. Immunol Rev **2009**; 229:216–31.
20. Ochsenbein AF, Riddell SR, Brown M, et al. CD27 expression promotes long-term survival of functional effector-memory CD8+ cytotoxic T lymphocytes in HIV-infected patients. J Exp Med **2004**; 200:1407–17.
21. Tanaskovic S, Price P, French MA, Fernandez S. Impaired upregulation of the costimulatory molecules, CD27 and CD28, on CD4+ T cells from HIV patients receiving ART is associated with poor proliferative responses. AIDS Res Hum Retroviruses **2017**; 33:101–9.
22. Huang J, Jochems C, Anderson AM, et al. Soluble CD27-pool in humans may contribute to T cell activation and tumor immunity. J Immunol **2013**; 190:6250–8.
23. Messele T, Brouwer M, Girma M, et al. Plasma levels of viro-immunological markers in HIV-infected and non-infected Ethiopians: correlation with cell surface activation markers. Clin Immunol **2001**; 98:212–9.
24. Shata MT, Abdel-Hameed EA, Hetta HF, Sherman KE. Immune activation in HIV/HCV-infected patients is associated with low-level expression of liver expressed antimicrobial peptide-2 (LEAP-2). J Clin Pathol **2013**; 66:967–75.
25. Kalayjian RC, Machekano RN, Rizk N, et al. Pretreatment levels of soluble cellular receptors and interleukin-6 are associated with HIV disease progression in subjects treated with highly active antiretroviral therapy. J Infect Dis **2010**; 201:1796–805.
26. Widney D, Gundapp G, Said JW, et al. Aberrant expression of CD27 and soluble CD27 (sCD27) in HIV infection and in AIDS-associated lymphoma. Clin Immunol **1999**; 93:114–23.
27. Yuan M, Ohishi M, Wang L, et al. Association between serum levels of soluble CD40/CD40 ligand and organ damage in hypertensive patients. Clin Exp Pharmacol Physiol **2010**; 37:848–51.
28. Mu CY, Qin PX, Qu QX, et al. Soluble CD40 in plasma and malignant pleural effusion with non-small cell lung cancer: a potential marker of prognosis. Chronic Dis Transl Med **2015**; 1:36–41.
29. Hock BD, Starling GC, Patton WN, et al. Identification of a circulating soluble form of CD80: levels in patients with hematological malignancies. Leuk Lymphoma **2004**; 45:2111–8.
30. Zhu X, Lang J. Soluble PD-1 and PD-L1: predictive and prognostic significance in cancer. Oncotarget **2017**; 8:97671–82.
31. Li N, Jilisihan B, Wang W, et al. Soluble LAG3 acts as a potential prognostic marker of gastric cancer and its positive correlation with CD8+T cell frequency and secretion of IL-12 and INF-γ in peripheral blood. Cancer Biomark **2018**; 23:341–51.
32. Burchill MA, Tamburini BA, Kedl RM. T cells compete by cleaving cell surface CD27 and blocking access to CD70-bearing APCs. Eur J Immunol **2015**; 45:3140–9.
33. Eshel D, Toporik A, Efrati T, et al. Characterization of natural human antagonistic soluble CD40 isoforms produced through alternative splicing. Mol Immunol **2008**; 46:250–7.
34. Fanslow WC, Anderson DM, Grabstein KH, et al. Soluble forms of CD40 inhibit biologic responses of human B cells. J Immunol **1992**; 149:655–60.
35. Horn LA, Long TM, Atkinson R, et al. Soluble CD80 protein delays tumor growth and promotes tumor-infiltrating lymphocytes. Cancer Immunol Res **2018**; 6:59–68.
36. Boyer Z, Palmer S. Targeting immune checkpoint molecules to eliminate latent HIV. Front Immunol **2018**; 9:2339.
37. Cheng L, Wang Q, Li G, et al. TLR3 agonist and CD40-targeting vaccination induces immune responses and reduces HIV-1 reservoirs. J Clin Invest **2018**; 128:4387–96.
38. Podlekareva D, Grint D, Karpov I, et al; EuroSIDA in EuroCoord. Changing utilization of Stavudine (d4T) in HIV-positive people in 2006-2013 in the EuroSIDA study. HIV Med **2015**; 16:533–43.
39. Raghavan A, Rimmelin DE, Fitch KV, Zanni MV. Sex differences in select noncommunicable HIV-associated comorbidities: exploring the role of systemic immune activation/inflammation. Curr HIV/AIDS Rep **2017**; 14:220–8.