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Changes in markers of T cell senescence and exhaustion with Atazanavir-, Raltegravir-, Darunavir-Based Initial Antiviral Therapy: ACTG 5260s

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ABSTRACT

It is unclear whether differential roles of CD4+ vs CD8+ T cell senescence/exhaustion and effects of antiretroviral therapy (ART) on these processes may contribute to morbidity in treated HIV-1 infection. In a prospective 96-week trial, 328 HIV-1-infected/ART-naïve participants were randomized to receive tenofovir-emtricitabine plus: atazanavir/ritonavir, darunavir/ritonavir, or raltegravir. Markers of CD4+ senescence (%CD28-CD57+) and CD4+/CD8+ T cell exhaustion (% PD1+) decreased after ART. There were no changes in markers of CD8+ T cell senescence after ART and no differential changes in all markers in ART groups. Senescent CD4+ and CD8+ T cells may have differential roles in HIV-1 pathogenesis.

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BACKGROUND

HIV-1 infection is characterized by a state of inflammation and immune activation that persists despite suppressive antiretroviral therapy (ART) and may contribute to the development of end-organ disease such as cardiovascular disease (CVD)[1]. Chronic HIV-1 infection is characterized by T cell senescence, a terminal state with dysregulated immune function and production of proinflammatory cytokines [2]. Senescent T cells may increase the risk of morbidity, such as CVD [3], but data in HIV-1 infection are limited and controversial [4, 5]. The mechanisms driving T cell exhaustion, immunosenescence, activation and chronic comorbidities in chronic HIV infection are not completely understood. In addition, although we and others have found that effective ART only partially decrease immune activation and inflammation [1], limited data exist on the effect of ART initiation in the setting of a randomized trial. Furthermore, it is unclear whether changes in these markers would differ in the setting of an integrase inhibitor-based regimen compared to a boosted protease inhibitor (PI) therapy. ACTG A5260s was a prospective cardiovascular substudy of A5257, in which participants were randomized equally to one of three regimens of tenofovir disoproxil fumarate-emtricitabine (TDF/FTC) plus an integrase-based regimen containing raltegravir (RAL) or a PI-based regimen containing either atazanavir/ritonavir (ATV/r) or darunavir/ritonavir (DRV/r) [1]. In this study we found that ATV/r was associated with slower progression of carotid intima-media thickness compared to DRV/r or RAL and that markers of inflammation and T-cell activation inconsistently and partially decreased after these regimens [1] without a consistent advantage for any of the regimens.

The objective of this analysis was to characterize the changes in biomarkers of T cell senescence longitudinally among treatment-naïve individuals initiating these randomized ART regimens. In this paper, we describe for the first time in the setting of a large randomized ART initiation trial, the effects of different successful ART regimens on T cell senescence and

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exhaustion. In addition, associations between key biomarkers of T cell exhaustion, immune senescence and activation, and inflammation were explored in ART-naïve HIV-infected participants at entry, and through 96 weeks of a successful ART regimen.

METHODS

Study Design and Participants

A5260s included 328 HIV-infected, ART-naïve adults with no CVD or diabetes mellitus or use of lipid-lowering medications. The design of this substudy has been previously reported [1], as well as key results related to cardiovascular and bone endpoints [6, 7]. The parent study and substudy (clinicalTrials.gov identifiers: NCT00811954, NCT00851799) were approved by the local Institutional Review Boards. For this pre-planned analysis, to avoid the confounding effect of viremia on markers of immunosenescence and exhaustion, the A5260s population was restricted to a subset of virologically suppressed participants who had no ART interruptions > 7 days and with HIV-1 RNA <50 copies/ml by study week 24 and thereafter.

Biomarker and Laboratory Assessment

Blood samples were drawn at study entry prior to ART initiation and after 24 and 96 weeks of treatment. Peripheral blood mononuclear cells (PBMCs) were isolated and cryopreserved and immunophenotyping was performed on these PBMCs using multicolor flow cytometry as previously [1, 6]. The fluorochrome-conjugated antibodies used were anti-CD3 PE-Cy7 (clone SK7), anti-CD4 V450 (clone RPA-T4), anti-CD8 APC (clone RPA-T8), anti-CD8 APC-Cy7 (clone SK1), anti-CD28 PE-Cy 5 (clone CD28.2), anti-CD57 PE (clone NK-1), anti- CD279 (PD-1) APC (clone MIH4), all from BD Biosciences. A representative gating strategy is shown in Supplemental Figure 1.

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Statistical Analyses

Changes over time were estimated as the mean difference of the on-treatment level compared to baseline on the log10 scale and back-transformed to represent mean fold change from baseline. For all pairwise treatment group comparisons, shifts in the distribution of change from baseline were evaluated using Wilcoxon rank sum tests and are described as relative fold-changes. While nominal p-values are presented, adjusted p-values using Benjamini-Hochberg methods to control the false discovery rate (FDR) are also provided to acknowledge comparisons across the multiple biomarkers at each study week. Exploratory bivariate associations, quantified with Spearman rank correlations, were examined at concurrent study weeks between levels and changes in biomarkers. All analyses were performed with SAS, version 9.4 (SAS Institute, Cary, North Carolina, USA).

RESULTS

Baseline characteristics

Baseline demographic characteristics of the 328 participants from the A5260s study were previously described [1]. The 234 participants (71%) included in the virologically suppressed population for this analysis had similar baseline demographic characteristics across treatment groups as previously described [1]. Briefly, 90% were men, 48% white, with a median age of 36 years, CD4+ cell count 338 cells/mm^3 and HIV-1 RNA 4.6 log10 copies/ml. Baseline levels of all biomarker parameters are presented in Table 1. No notable differences in distributions are apparent between treatment groups.

Changes in markers of immunosenescence and exhaustion

Sustained decreases (about 31% or more) from baseline in levels of % CD28-CD57+ of CD4+ T cells were evident among all treatment groups (Figure 1, Table 1). In contrast, there was no apparent change from baseline to 96 weeks in levels of % CD28-CD57+ of CD8+ T cells. For

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exhaustion markers, declines from baseline were evident for % PD1+ of both CD4+ and CD8+ T cells across all treatment groups, by week 24, with further apparent declines for CD4 + T cell exhaustion by week 96; week 96 levels were on average at least 56% lower than baseline levels. Treatment group differences were not apparent for any immunosenescence and exhaustion marker (p>0.1;Supplemental Table 1). Overall, sustained declines over time were evident in all treatment groups for CD4+ T cell markers of senescence and exhaustion.

Correlations between biomarkers of T cell senescence, exhaustion, inflammation and immune activation

At baseline, plasma viral load was correlated with markers of exhaustion (PD1+) (r=0.24-0.31; p<0.001), but not senescence (|r|<0.15, p>0.05). With regards to associations of baseline markers of immunosenescence with markers of T-cell and monocyte activation, or inflammation at baseline (Supplemental Table 2), the strongest associations were apparent between % of CD4+ CD38+DR+ and % of CD4+ CD28-CD57+ (r=0.42) and % of CD4+ PD1+(r=0.40) (Supplemental Figure 2). There was also a moderate association at baseline between monocyte activation (sCD163) and CD4+ exhaustion (% of CD4+ PD1+) (r=0.35). These moderate associations were also apparent when examining the concurrent changes over 96 weeks between these set of markers with correlations ranging from 0.33 to 0.55 (Supplemental Table 3); in contrast, a moderate association between the levels of these markers at week 96 was only apparent for CD4+ activation and senescence (r=0.44). All remaining associations between biomarkers were not apparent or weak (|r|<0.3) (Supplemental Tables 2, 3)

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DISCUSSION

In this prospective study of ART-naïve participants who achieved virologic suppression after initiation of TDF/FTC along with RAL, ATV/r or DRV/r, RAL did not have a more favorable effect on decreasing immunosenescence or exhaustion compared to the PIs. To our knowledge, this is the largest prospective study describing changes in markers of immune senescence and exhaustion after initiation of successful ART. We also describe associations with markers of immune activation and inflammation that have been associated with serious clinical events in HIV-1 infected persons, including CVD and mortality [8]. Of note, although sustained and similar declines over time from baseline were evident in all treatment groups for all CD4+ T cell markers of senescence and exhaustion, consistent reductions in markers of CD8+ T cell senescence were not apparent. Overall, these results add to the current literature outlining the incomplete reversal of inflammation, senescence and immune activation in the setting of effective treatment [9].

Understanding how different initial ART regimens reduce chronic immune dysfunction in successfully treated HIV-1 infection is an ongoing research priority. Other studies have suggested that integrase inhibitors may reduce inflammation and viral load more effectively than other antiretroviral agents[10][11]. Intensification with the integrase inhibitor RAL has shown variable effects on immune activation[10]. Despite this limited evidence, the differential effects of integrase inhibitors compared to other ART on T cell senescence remain unknown. We did not find any benefit of RAL over PIs on reducing immunosenescence or exhaustion during the first 96 weeks of successful treatment. This is consistent with a study by Kaplan et al, in which no differential effect of PIs were seen compared to NNRTIs on T cell senescence [4]. A strength of our study is that, in contrast to prior RAL switch or intensification-related studies, our data are not confounded by prior ART.

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The age-associated remodeling of the immune system, through accumulation of senescent T cells may contribute to numerous aging-related pathologies [2]. Senescent T cells have been associated with CVD [3] but the differential role of CD4+ compared to CD8+ T cell senescence in development of chronic comorbidities such as CVD and bone disease remain unclear. CD8+ T cell senescence has been associated with progression of numerous diseases [2] including CVD in a study of HIV-infected patients [4] but this was not confirmed by others [5]. Senescent CD4+ T cells are rare long-lived senescent T cells with proatherogenic properties [12] that may result from persistent antigenic stimulation[12] and play a critical role in the development of age-related pathologies [2]. HIV-infected patients with low-level CD4+ T cell count on ART have more senescent CD4+ T cells compared to those with increased CD4+ T cell levels [13]. The lack of change in markers of CD8+ T cell senescence in our study may be due to our cohort having overall a relatively high pre-ART CD4+ T cell count and may be overall lower pre-ART levels of senescent CD8+ T cells than other studies [4, 5, 9]. Together, these prior studies highlight possible differential roles of senescent CD4+ versus CD8+ T cells [14] in the pathogenesis of HIV and its comorbidities, and are consistent with our results that effective ART reduced differentially CD4+ (and not CD8+) T cell senescence.

In view of the limited data regarding the interplay between T cell exhaustion, senescence, inflammation and immune activation in chronic HIV infection, we found modest bivariate associations only between biomarkers of CD4+ and CD8+ T cell activation and exhaustion at baseline and change over 96 weeks. At week 96, these associations were apparent for CD4+ activation and exhaustion biomarkers but not CD8+. The only other moderate association detected was between baseline biomarkers of CD8+ T cell exhaustion and sCD163, a biomarker of monocyte activation that has been associated with CVD [15]. This finding may be explained by the fact that normal CD4 T cells orchestrate multiple signals from T-cells, B-cells, and antigen presenting cells and that senescent CD4+ T cells contribute to the pathogenesis of

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states of immune activation such as autoimmunity [2]. Further studies are needed to elucidate the cross talk between HIV infection, T cell activation, T cell senescence and exhaustion and chronic comorbidities such as CVD and bone disease.

Our study had several limitations that have previously been described. Briefly these include limited power to detect effect sizes with adjustment for multiple biomarker comparisons, selection bias of A5260s participants when restricting to the cohort of virologically suppressed individuals who remained on ART, inclusion of mostly men, which may limit generalizability of our findings. Finally, the relatively younger age of our study population with no CVD may have limited our ability to study the cross talk between T cell senescence, exhaustion and immune activation.

In conclusion, in this prospective study we did not find differential changes in T cell senescence and exhaustion after 96 weeks among treatment-naïve individuals initiating and remaining on successful ART regimens of TDF/FTC with RAL, ATV/r or DRV/r. Despite successful ART therapy, markers of CD8+ T cell senescence did not decline; increased CD4+ T cell activation was associated with increased CD4 T cell exhaustion and senescence. Overall, these data suggest differential roles of senescent CD4+ and CD8+ T cells in HIV immunopathogenesis in the setting of effective ART. Furthermore, these results also highlight the need to further understand the role of T cell senescence and exhaustion in chronic HIV infection that may lead to adjunct ART regimens with more potent anti-inflammatory effects as a strategy to prevent chronic comorbidities associated with HIV-1 infection.

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NOTES

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Authors contributions: J.C, J.S., G.M., T.B, T.K. were responsible for the study concept and design. T.T, C.M, H.R carried out the statistical analyses. T.K.,T.T, C.M, G.M drafted the manuscript. T.K., O.Y, M.D, J.S., J.C., G.M. T.B. collected the data. All co-authors participated

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in discussions about the design of the study, interpretation of the findings, and critically reviewed the manuscript.

Potential conflicts of interest: Dr Brown has served as a consultant for BMS, GSK, Merck, Abbott, Gilead, ViiV Healthcare and has received research funding from Merck and GSK. Dr. Currier has served as a consultant for Gilead and has received research funding from Merck. Dr Stein served on a Data Safety Monitoring Board for Lilly. Dr McComsey has served as consultant or received research grants from BMS, Pfizer, Gilead, ICON, and GSK/ViiV. Dr Ribaudo, Dr. Moser and Thuy Tran have no Duality of Interest disclosures. Dr. Dubé has served as a consultant for Gilead and Astra Zeneca, and receives research funding from Gilead, ViiV, and Merck.

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Table 1. Baseline values for study participants and mean fold change (95% CI) from baseline over time by treatment group

	Total (N=212)	ATV/r (N=64)		RAL (N=72)		DRV/r (N=76)	
Biomarkers	**Baseline (n=58)**	**Week 24 (n=58)**	**Week 96 (n=61)**	**Week 24 (n=65)**	**Week 96 (n=70)**	**Week 24 (n=73)**	**Week 96 (n=72)**
Immunosene scence							
%CD8+: CD28-CD57+	24.35 (17.8,30.75)	1.04 (0.91,1.19)	0.96 (0.85,1.09)	***1.15 (1.07,1.23)***	1.07 (0.98,1.17)	***1.13 (1.07,1.20)***	0.99 (0.89,1.10)
%CD4+: CD28-CD57+	5.01 (2.24,9.97)	***0.73 (0.60,0.90)***	***0.66 (0.52,0.85)***	***0.85 (0.72,1.00)***	***0.76 (0.62,0.93)***	***0.85 (0.74,0.97)***	***0.65 (0.54,0.79)***
Cell exhaustion							
%CD8+: PD1+	2.33 (1.48,3.87)	***0.43 (0.34,0.55)***	***0.42 (0.33,0.54)***	***0.42 (0.36,0.49)***	***0.33 (0.27,0.40)***	***0.35 (0.30,0.42)***	***0.32 (0.24,0.43)***
%CD4+: PD1+	4.37 (2.57,7.62)	***0.58 (0.48,0.70)***	***0.38 (0.30,0.47)***	***0.60 (0.50,0.70)***	***0.44 (0.37,0.52)***	***0.50 (0.44,0.58)***	***0.39 (0.32,0.48)***

n gives the number of participants with baseline and follow markers available for calculation of change. Significant (95% CI not including 1.0) results are marked in bold.

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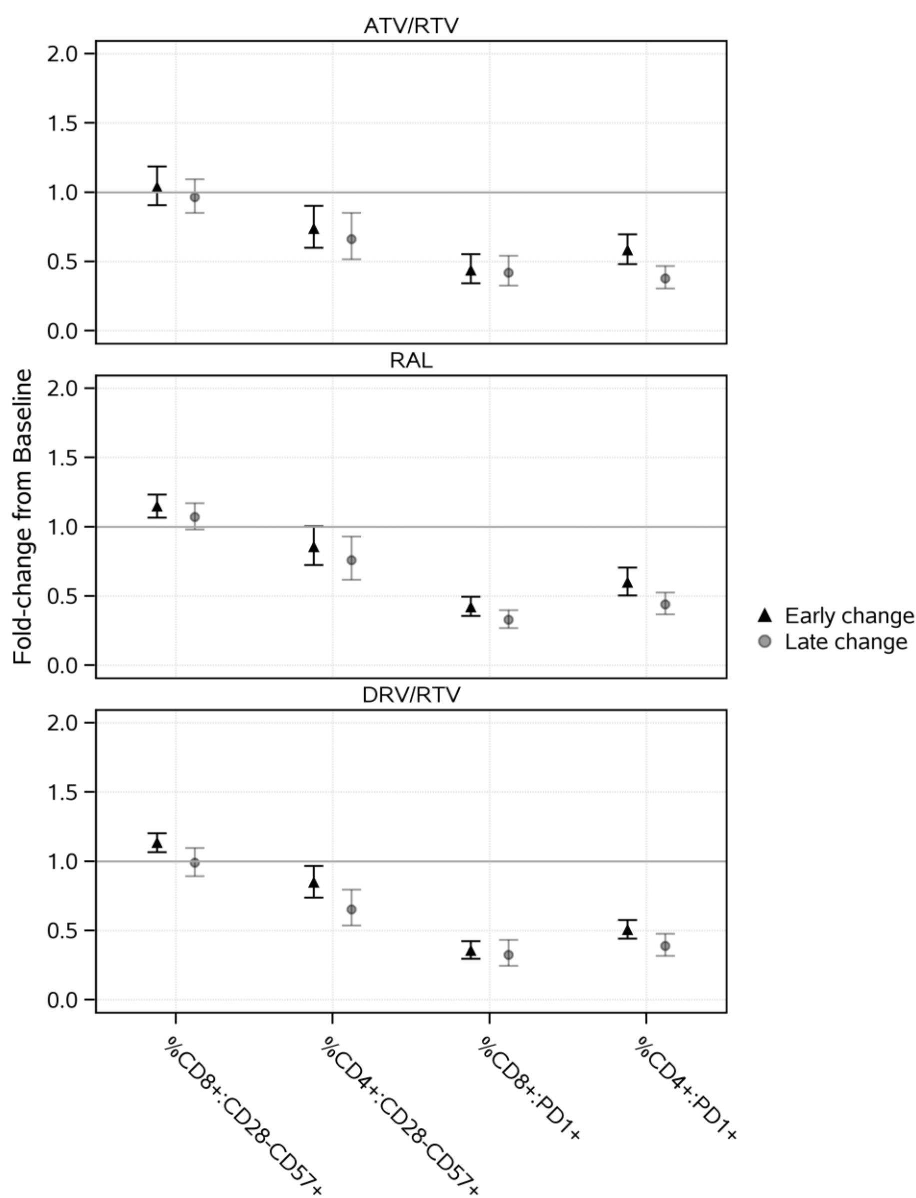

FIGURE LEGENDS

Figure 1. Early and Late Changes in Immunosenescence Markers by Treatment Arm

Point estimates and error bars reflect mean and 95% confidence intervals, respectively.
Early change represents change from baseline to week 24; late change represents change from baseline to week 96.
ATV/r = Atazanavir/Ritonavir, DRV/r = Darunavir/ Ritonavir, RAL = Raltegravir

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