Different impact of raltegravir versus efavirenz on CD4/CD8 ratio recovery in HIV-infected patients

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Received 19 May 2016; returned 6 July 2016; revised 10 August 2016; accepted 10 August 2016

Objectives: A low CD4/CD8 ratio during treated HIV identifies individuals with heightened immunooactivation and excess mortality. Whether ART regimens elicit distinct CD4/CD8 ratio recovery remains unknown. We aimed to compare the efficacy of an integrase inhibitor versus a non-nucleoside to normalize the CD4/CD8 ratio.

Methods: We conducted a post hoc analysis of the STARTMRK study, a randomized, blinded, double-dummy Phase III trial of raltegravir versus efavirenz, and each in combination with tenofovir/emtricitabine, in treatment-naïve HIV-infected adults. Blinding was maintained for the entire 5 year duration of the study. Kaplan–Meier methods for time-dependent variables were used to calculate the rates of CD4/CD8 normalization at different cut-offs and cumulative probabilities. Cox proportional hazard models were used to compare probabilities of CD4/CD8 normalization by treatment arm.

Results: A total of 563 patients were analysed; 81% were males and the mean age (SD) was 37 (10) years. Raltegravir was associated with higher rates of CD4/CD8 ratio normalization at the >0.4 cut-off (median time to normalization = 56 versus 84 days; \( P = 0.048 \) by log-rank test). A Cox proportional hazard model stratified based on baseline CD4 counts showed an association between raltegravir and higher rates of CD4/CD8 ratio normalization \( (HR = 1.23; P = 0.02) \).

Conclusions: We herein show that normalization of the CD4/CD8 ratio above a clinically meaningful threshold may be dependent on the drug class used. Raltegravir showed faster CD4/CD8 ratio normalization compared with efavirenz, a finding with potential clinical implications. Whether other integrase inhibitors have a similar impact for this outcome remains to be explored.

Introduction

The CD4/CD8 ratio has emerged as a novel biomarker for HIV disease. Mounting evidence suggests that a low CD4/CD8 ratio, driven by persistence of heightened CD8+ T cell counts during effective ART, correlates with persistent immune defects of the innate (e.g. the kynurenine pathway) and adaptive immunity (e.g. T cell activation and senescence). Importantly, while each of these immunological markers predict mortality, persistence of a low CD4/CD8 ratio and/or increased numbers of circulating CD8+ T cells have also been associated with poor prognosis in several cohort studies. A CD4/CD8 ratio >1 has been proposed as the cut-off to define normal values in the general population. The studies establishing a link between a low CD4/CD8 ratio and mortality during treated infection, however, suggest that lower values, between 0.3 and 0.5, might more accurately identify subjects at risk.

While data on the biological and clinical significance of a low CD4/CD8 ratio during ART are accumulating, this biomarker, readily available in routine clinical practice, has been largely overlooked in prior studies assessing the impact of therapies to correct the immune abnormalities elicited by HIV infection. For example, beyond a clear benefit of early ART initiation on the rate of CD4/CD8 ratio recovery, it is unknown whether current first-line ART regimens differ in the rates of CD4/CD8 ratio normalization, which could ultimately improve prognosis. Recent preliminary data have suggested that integrase inhibitors might reduce immune dysfunction compared with NNRTI- and PI-based regimens. Hence, we aimed to compare the effects of raltegravir-based versus efavirenz-based therapy on the CD4/CD8 ratio using the final 5 year data from a Phase III non-inferiority trial in treatment-naïve patients (STARTMRK), which remained blinded until its conclusion at 5 years.

Methods

The STARTMRK trial (MK-0518 Protocol 021) was a blinded, double-dummy, randomized, active control, Phase III clinical trial originally planned for 96 weeks, but extended to 240 weeks. The details of the
study have been described elsewhere. Briefly, HIV-untreated patients without baseline genotypic resistance to efavirenz, tenofovir or emtricitabine were eligible. Yearly analyses were planned, with primary and secondary endpoints stipulated at weeks 48 and 96, respectively. Blinding was maintained for the entire duration of the study. Yearly analyses were planned, with the primary analysis at week 48, secondary analysis at week 96 and exploratory analyses at weeks 156, 192 and 240.

**Ethics**

The protocol was approved by the appropriate review committee at each site and executed in accordance with Good Clinical Practice guidelines. All participants provided written informed consent.

**Statistical methods**

All randomized and treated patients in STARTMRK were included in this exploratory post hoc analysis. The outcome variable was the time to CD4/CD8 normalization at cut-offs (>0.4, >1, >1.5 and >2.0). The rationale for this categorization was: (i) values between 0.3 and 0.5 have been associated with clinical progression in treated patients, with 0.4 as the cut-off with the best predictive value; (ii) values <1 have been associated with features of immunosenescence in the general population and HIV-infected patients above this cut-off appear to have similar levels of immunoreactivation and immunosenescence than healthy controls; and (iii) normal CD4/CD8 ratio values in young individuals range between 1.5 and 2. Although additional cut-offs were tentatively explored (>0.5, >0.8, >1.2) the results did not add meaningful information and lacked the rationale of the other cut-offs. For each of these cut-offs, Kaplan–Meier methods for time-dependent variables were used to calculate the rate of CD4/CD8 normalization and cumulative probabilities. Cox proportional hazard models were used to compare the time to CD4/CD8 normalization by treatment arm. We validated the proportional hazard assumption by testing time-dependent variables and graphs based on the Schoenfeld residuals. Given the rapid effect of integrase inhibitors on the normalization of HIV-RNA, we adjusted for the baseline HIV-RNA (<10^5 versus ≥10^5 copies/mL) with or without the interaction effect between treatment and baseline stratifications. Since the interaction term was not significant, it was excluded from the final adjusted model for baseline HIV-RNA. Given the reported association between pretreatment CD4 counts and CD4/CD8 ratio normalization, further statistical analyses were further adjusted by the baseline CD4+ T cell counts. Since this variable did not meet the proportional hazards assumption, we adjusted for it by a stratified Cox proportional hazard model. To assess the effect of CD4+ and CD8+ T cell count changes on the overall CD4/CD8 ratio dynamics we fitted a linear mixed model, defining the CD4/CD8 ratio as the response variable and adjusting for the CD4+ and CD8+ T cell counts as well as treatment group and duration on therapy. All P values reported are not adjusted for multiple testing and should be viewed as hypothesis generating only.

**Results**

**Characteristics of the study population and duration of follow-up**

A total of 563 patients were randomized to raltegravir (n = 281) or efavirenz (n = 282) and were included in the analysis. The baseline characteristics for patients are summarized in Table 1 (available as Supplementary data at JAC Online). The study sample was representative of a medium-aged (37.2 ± 9.5 years) population with higher representation of men (81.3%) initiating ART at low CD4+ T cell counts [208 (140–284) cells/mm^3]. The two groups were balanced for all parameters. Discontinuations due to adverse events occurred in 14 (5%) and 28 (10%) patients in the respective groups, as previously described. Thirty-four patients were lost to follow-up, with no between-group differences. The median follow-up of the 563 patients was 55 months.

**Changes in CD4/CD8 ratio normalization per treatment arm**

The overall longitudinal changes in the CD4/CD8 ratio are represented in Figure S1. The time to CD4/CD8 normalization of raltegravir and efavirenz at cut-offs of >0.4, >1, >1.5 and >2.0 using Kaplan–Meier methods is summarized in Table 1. Comparison between treatment groups in the time to CD4/CD8 normalization of cut-offs of >1, >1.5 and >2.0 showed no statistically significant difference (P > 0.05 by log-rank test). We found, however, a statistically significant difference between the two treatment arms with respect to the time to CD4/CD8 normalization at cut-off >0.4. The proportion of patients whose CD4/CD8 ratio remained below 0.4 during treatment is summarized in Table S2, showing that by week 240, <5% of patients showed values below this cut-off, with no statistically significant differences between treatment arms. Next, we explored the time to CD4/CD8 ratio at cut-off >0.4 in proportional hazards models (see Table S3). In the model adjusted for baseline HIV-RNA, the time to CD4/CD8 normalization >0.4 was similar between the two treatment arms, but trending in favour of the raltegravir group (P = 0.07). Interestingly, baseline HIV-RNA <10^5 copies/mL was a predictor of faster normalization >0.4 compared with baseline HIV-RNA ≥10^5 copies/mL (HR = 1.63; 95% CI = 1.37–1.95; P < 0.001), yet no significant interaction between treatment arm and baseline HIV-RNA was detected (Figure S2 and Table S3). We then adjusted the analyses by the baseline CD4 counts (<200, 201–500 and >500 cells/mm^3) in a stratified Cox proportional hazard model (Figure S3 and Table S3). We observed a significantly shorter time to CD4/
CD8 normalization with raltegravir compared with efavirenz (HR = 1.23; 95% CI = 1.03–1.46; P = 0.02). These findings were not reproduced for CD4/CD8 ratio cut-offs >1, >1.5 and >2.0, for which no significant differences of raltegravir compared with efavirenz were detected (see Figures S4 to S6 for Kaplan–Meier survival plots of estimated overall 5 year CD4/CD8 normalization at different cut-offs).

Finally, we examined in a linear mixed effect model whether the overall CD4/CD8 ratio increase was driven by increases in the CD4+ T cell counts, decreases in the CD8+ T cell counts or both. We observed that changes in both CD4+ and CD8+ T cell counts significantly contributed to the mean CD4/CD8 ratio increase (Table S4). To characterize further we calculated the mean differences of each parameter over time (Table S5).

**Discussion**

Using data from a randomized, double-blinded clinical trial with 5 year follow-up we herein show, for the first time to our knowledge, a significantly faster time to CD4/CD8 ratio normalization (>0.4) with raltegravir-based compared with efavirenz-based first-line ART. Recent preliminary data suggested that switch or intensification ART strategies with integrase inhibitors might reduce immune dysfunction compared with NNRTI- and PI-based regimens. 12–14,19 Moreover, in a single centre cohort an independent association between raltegravir and CD4/CD8 ratio normalization was described, 15 and raltegravir caused a significant CD4/CD8 increase when added to suppressive ART in a pilot study in patients with poor CD4+ T cell increase. 20

We only observed differences between raltegravir versus efavirenz for CD4/CD8 normalization at the >0.4 cut-off. We have previously described in a study including treated patients with CD4+ T cell counts >500/mm³ an association between CD4/CD8 ratios <0.4 and prominent T cell activation and senescence. In contrast, subjects with ratios >1.0 displayed traits of a healthy immune system. 1 Importantly, only values between 0.3 and 0.5 have so far demonstrated to predict mortality in HIV-infected individuals on ART, 1,5,7,8 and 0.4 has been proposed as the cut-off with the best diagnostic accuracy as a predictor of severe non-AIDS illnesses in one study. 5 The clinical implications of persistence of ratios below other cut-offs (i.e. 1, 1.5 and 2.0) in this setting remain unknown, although conceivably reflect subtle underlying immune abnormalities with unclear impact on long-term prognosis. Beyond clinical outcomes, persistently low CD4/CD8 ratios during ART seem to be predictive of impaired...
vaccine response\textsuperscript{21,22} and subclinical disease, including increased peripheral fat,\textsuperscript{23} sarcopenia,\textsuperscript{24} carotid atherosclerosis\textsuperscript{24–26} and impaired kidney function.\textsuperscript{26} Therefore, our data suggest greater efficacy of raltegravir to restore the CD4/CD8 ratio above a clinically meaningful threshold. Despite the long follow-up period, our study was not powered to evaluate the impact of the CD4/CD8 ratio and the study intervention on the risk of non-AIDS events. It is unlikely that clinical trials powered to detect differences between ART regimens evaluating the development of non-AIDS comorbidities will be performed in the future, given the high rates of virological control with current regimens and the overall low risk of disease. Hence, studies evaluating the impact of therapies on surrogate markers of non-AIDS-defining conditions are needed to infer differences on long-term outcomes and are increasingly being reported in the HIV literature. Revisiting the data from key clinical trials offers a unique opportunity to gain insights on the effects of ART on the CD4/CD8 ratio.

Our findings highlight the potential of raltegravir-based regimens to restore the immune system beyond the peripheral CD4+ T cell counts, in keeping with previous observations,\textsuperscript{12–14,19} and provide new support to the concept that treatments based on integrase inhibitors might prove more potent to normalize residual immune defects and, ultimately, impact on long-term mortality. Of note, this effect seems specific of patients with more advanced disease, as determined by a low CD4/CD8 ratio (<0.4). The increase of CD4/CD8 ratio recovery was influenced by both the increase of CD4+ and a decrease of CD8+ T cell counts. Over time, the magnitude of increase in mean CD4+ T cell count was larger than the respective magnitude of decline in mean CD8+ T cell count. This might be influenced by the fact that the STARTMRK participants were characterized by advanced HIV disease at baseline (mean CD4+ T cell counts, 207 cells/µL). Whether any ART regimen might prove more potent to normalize the CD8+ T cell counts remains an open question and might require evaluation of less advanced patients, in which a low ratio is usually driven by heightened CD8+ T cell counts. Interestingly, a subanalysis of the START trial has recently shown that even among subjects with baseline CD4 counts >500 cells/µL, a low CD4/CD8 ratio (<0.5) identifies a subgroup of patients at advanced risk of disease, which benefited most from early ART initiation,\textsuperscript{18} emphasizing the need of specific interventions in this clinical phenotype of patients.

Given the emerging evidence suggesting that patients with persistently CD4/CD8 ratios despite optimal HIV-RNA suppression might benefit from specific interventions, we believe that this study has clinical implications and reinforces the positioning of integrase inhibitor-based therapies with respect to NNRTI- or PI-based regimens as first choice. The impact of other integrase inhibitors on CD4/CD8 ratio recovery, however, must still be examined before assuming a drug class effect. Evaluation of the CD4/CD8 ratio in future trials of therapeutic interventions in HIV will allow the field to move forward.

Acknowledgements

We thank all the patients and their caregivers who participated in this STARTMRK study. The contributions of the many investigators are gratefully recognized.

Funding

The studies included in this report were sponsored and funded by Merck, which manufactures raltegravir under the brand name ISENTRESS\textsuperscript{6}. S. S.-V. is funded by a grant of the Spanish Ministry of Economy and Competitiveness (Contratos Juan Rodés JR14/0004).

Transparency declarations

S. S.-V. reports personal fees from ViV, MSD, Gilead and Janssen, outside the submitted work. Y. Z. and A. J. R. are employed by Merck. S. M. reports grants from ViV, MSD and Gilead, and personal fees from Abbvie, BMS, ViV, Gilead and MSD, outside the submitted work.

Supplementary data

Tables S1 to S5 and Figures S1 to S6 are available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

References

4 Mudd JC, Lederman MM. CD8 T cell persistence in treated HIV infection. JAC 2016; 72: 235–42.


