

jurisdictions considering replicating the MCC program. Of the remainder, some costs related to protocol and assessment tool development could potentially be minimized by adapting materials already developed for LAC MCC. The annual cost per patient was \$1100–\$3100; although the annual costs of similar programs vary widely,<sup>6–8</sup> it is within the range reported for these similar programs and is also comparable with the cost of a 1-month supply of a single-tablet coformulated antiretroviral therapy medication.<sup>9</sup>

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**OPEN**

# The Effect of Switching to Maraviroc + Darunavir/Ritonavir Dual Therapy in Virologically Suppressed Patients on the Progression of Liver Fibrosis: Findings From a Randomized Study

In vitro and animal studies revealed a potential protective role of CCR5 antagonists on reducing liver fibrosis progression and protecting from developing hepatocellular carcinoma.<sup>1</sup> Hepatocytes bear CXCR4 and CCR5, the 2 main coreceptors for HIV entry into cells and the blockade of coreceptors on hepatic stellate cells, the major producers of extracellular matrix in the liver, will slow progression of liver fibrosis, especially due to HIV-envelope gp120-mediated fibrogenesis modulation.<sup>2–5</sup>

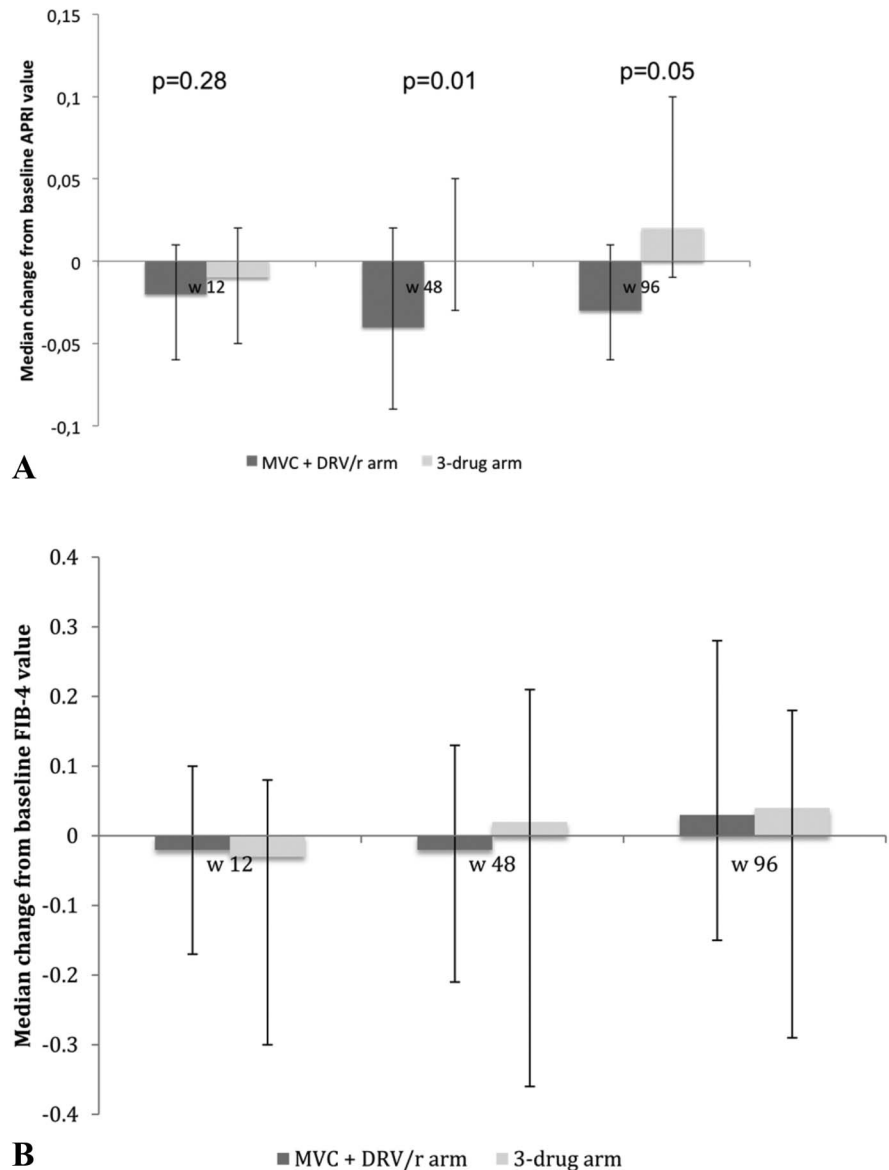
The aim of present analysis was to compare the evolution of liver fibrosis over time evaluated by surrogated biomarker assays in HIV-1-infected patients on a virologically successful

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**FIGURE 1.** A, APRI score during follow-up. B, FIB-4 during follow-up. No significant difference between arms at each time-point.

antiretroviral therapy (stable HIV-1 RNA <50 copies/mL), randomized to switch to maraviroc + darunavir/r (MVC + DRV/r arm) qd or to continue the current MVC-free 3-drug antiretroviral therapy (ART) (3-drug ART arm).

Patients included in the study were enrolled in the GUiDed Simplification with Tropism Assay (GUSTA) trial, a multicenter, open-label, randomized study ([www.clinicaltrials.gov](http://www.clinicaltrials.gov), number NCT01367210), whose main results have been published.<sup>6</sup>

Briefly, GUSTA included patients with HIV-1 RNA <50 copies/mL for at least 6 months, R5 tropism and CD4 counts >200 cells/ $\mu$ L for at least 3

months before enrollment; hepatitis B virus–coinfected patients and those with Child-Pugh B/C cirrhosis were excluded.

We retrospectively evaluated Fibrosis-4 (FIB-4) Index and aspartate aminotransferase to Platelet Ratio Index (APRI) scores, at baseline and after 12, 24, 48, and 96 weeks. The cutoff points of serum marker tests of hepatic fibrosis were as follows: FIB-4 <1.45 (F0-F1), 1.45–3.25 (indeterminate), and >3.25 (F3-F4); APRI <0.5 (F0-F1), >1.5 (F2) and >2 (cirrhosis).

Differences between arms were assessed by  $\chi^2$  and Student *t* test, longitudinal within-group differences

by McNemar test. The FIB-4 Index and APRI scores were used as continuous variables; their predictors at baseline and their change over time were investigated by linear regression.

We included 150 patients, 76 randomized to MVC + DRV/r arm and 74 to 3-drug ART arm. Baseline characteristics were homogeneous between arms except for relative younger age in the MVC + DRV/r arm (median 47 yrs; interquartile range [IQR] 40–52) than in the 3-drug ART arm (50 yrs; IQR 44–57) (*P* = 0.08), more frequent African ethnicity in the 3-drug ART arm than in the MVC + DRV/r arm (8% vs. 1%) (*P* = 0.05), and FIB-4 median value

higher in the MVC + DRV/r arm (1.15; IQR 0.82–1.32) than in the 3-drug ART arm (0.91; IQR 0.68–1.20) ( $P = 0.01$ ). APRI score was similar between arms: 0.23 (IQR 0.18–0.29) in the MVC + DRV/r arm and 0.25 (IQR 0.20–0.33) in the 3-drug ART arm ( $P = 0.12$ ).

Overall, 89% (134/150) were males and Caucasians; 41% (61/150) were heterosexuals; 38% (57/150) homosexuals/bisexuals; 7% (10/150) reported history of injected drug use, 11 years of HIV (IQR 7–18), 10 years of ART (IQR 6–15), CD4 at nadir 222 cells/mm<sup>3</sup> (IQR 132–319) and at baseline 654 cells/mm<sup>3</sup> (IQR 506–905). Eighteen patients presented positive serology for hepatitis C virus (HCV) and 8 had a detectable HCV RNA, 4 in each arms.

Sixteen (11%) presented diabetes mellitus: 12% (9/76) in the MVC + DRV/r arm and 9% (7/74) in the 3-drug ART arm ( $P = 0.04$ ). At screening, nucleoside reverse transcriptase inhibitors (NRTIs) were used in 95% (143/150), nonnucleoside reverse transcriptase inhibitors (NNRTIs) in 12% (18/150), integrase strand transfer inhibitors (INSTIs) in 18% (17/150), and protease inhibitors (PIs) in 69% (103/150) of which boosted PI in 63% (94/150) and DRV/r in 31% (47/150). No differences between arms were observed in terms of dyslipidemia (in 100/150, 66%), with total cholesterol 203 mg/dL (IQR 173–230), body mass index (23 kg/m<sup>2</sup>, IQR 22–26) and glucose 89 mg/dL (IQR 82–100). Median value of false positive rate at geno2pheno was 43 (IQR 24–69), with no differences between groups.

During observation in the 3-drug ART arm ( $n = 74$ ), NRTIs were used in 92%, NNRTIs in 16%, INSTIs in 15%, PIs in 69%, boosted PI in 51%, and DRV/r in 43%.

According to the cutoff points of hepatic fibrosis, FIB-4 in the MVC + DRV/r arm was <1.45 in 83% (63/76), between 1.45 and 3.25 in 16% (12/76), and >3.25 in 1% (1/76); in the 3-drug ART arm, it was <1.45 in 88% (65/74) and between 1.45 and 3.25 in 12% (9/74) (no one had FIB-4 >3.25).

Overall, APRI was <0.5 in 91% (137/150), and no one had >1.5 at baseline.

Based on the FIB-4 score, at 48 weeks progression to a higher level was observed in 6% (4/63) in the MVC +

DRV/r arm and in 6% (4/65) in 3-drug ART arm; in 3% (4/12) among those in MVC + DRV/r arm and in 3% (3/9) in 3-drug ART arm, FIB-4 improved by at least 1 stage, whereas the other patients did not modify their FIB-4 stratum.

Based on the APRI score, at 48 weeks, significant modification of the stratum was no observed.

In addition, no significant differences between arms were observed in platelet counts and alanine transaminase changes at 48 weeks from baseline. We observed a more profound decrease of aspartate transaminase (AST) levels in the MVC + DRV/r arm (mean change  $-4.19$  IU/L, SD 7.2) vs. 3-drug ART arm (mean change  $+0.58$  IU/L, SD 9.9) ( $P = 0.007$ ).

In a multivariable model adjusting for risk factor for HIV acquisition and duration of ART exposure, longer time from HIV diagnosis (per 1 year increase  $+0.031$ , 95% confidence interval [CI]:  $+0.007$  to  $+0.055$ ,  $P = 0.01$ ), lower nadir CD4<sup>+</sup> cells count ( $+100$  cells increase,  $-0.060$ , 95% CI  $-0.107$  to  $-0.014$ ,  $P = 0.01$ ), and HCV antibody positive status ( $+0.321$ , 95% CI  $+0.000$  to  $+0.642$ ,  $P = 0.05$ ) were associated with higher baseline FIB-4 values. No factor independently associated with baseline APRI values was observed. During follow-up, the APRI score decreased more prominently in the MVC + DRV/r arm vs 3-drug ART arm at week 12 (median change  $-0.02$ ; IQR  $-0.06$  to  $+0.12$  vs  $-0.006$ ; IQR  $-0.05$  to  $+0.02$ ;  $P = 0.28$ ), at week 48 ( $-0.04$ ; IQR  $-0.09$  to  $+0.02$  vs  $+0.001$ ; IQR  $-0.037$  to  $+0.049$ ;  $P = 0.01$ ), and at week 96 ( $-0.03$ ; IQR  $-0.06$  to  $+0.01$  vs  $+0.02$ ; IQR  $-0.01$  to  $+0.10$ ;  $P = 0.053$ ) (Fig. 1A).

In a multivariable model, predictors of APRI change at 48 weeks were baseline APRI ( $-0.391$ ; 95% CI  $-0.515$  to  $-0.266$ ;  $P < 0.001$ ) and MVC + DRV/r arm vs 3-drug ART arm ( $-0.040$ ; 95% CI  $-0.006$  to  $-0.074$ ;  $P = 0.021$ ).

FIB-4 also showed a trend toward a more prominent reduction in the MVC + DRV/r arm ( $-0.02$ ; IQR  $-0.21$  to  $+0.13$ ) vs 3-drug ART arm ( $+0.02$ ; IQR  $-0.23$  to  $+0.20$ ) ( $P = 0.35$ ) at week 48 (Fig. 1B). Baseline FIB-4, but not study arm, predicted FIB-4 modifications during follow-up.

In conclusion, we observed that switch to MVC + DRV/r in HIV-1-infected, but virologically suppressed

patients on 3-drug ART, was associated with a slight but significant improvement of the APRI score over time as compared with continuing 3-drug ART without MVC. This MVC-containing regimen did not significantly influence the longitudinal change of the FIB-4 score, possibly due to the presence of age as a component of the score, which was increasing over time in the study patients, although a trend toward an improvement was observed. Our observations are in agreement with experiments showing a reduction of hepatic stellate cells activation and fibrosis progression and an improved survival in a murine model of hepatocellular carcinoma<sup>1</sup> and in vitro observations on the inhibitory effect of MVC on the accumulation of fibrillar collagens and extracellular matrix proteins by human hepatic stellate cells.<sup>7</sup> Results from this study are also in line with a previous retrospective non-comparative analysis on 71 HIV/HCV-coinfected patients treated with MVC, showing a potential beneficial effect on liver fibrosis measured by the APRI score.<sup>8</sup> In a previous prospective, non-controlled pilot study on 24 HIV/HCV-coinfected patients starting a MVC-based regimen, liver fibrosis was slightly but not significantly reduced, although observation was limited to 6 months.<sup>9</sup> In addition, a recent study suggests that a validated marker of liver fibrosis was reduced in HIV-1-infected patients carrying the variant allele CCR5 delta-32, associated with reduced CCR5 expression, and in patients exposed to cenicriviroc, a CCR5/CCR2 blockade agent.<sup>10</sup>

Our study adds to previous evidence and has its strengths in the randomized comparison, the study arm treated with a homogeneous MVC-containing regimen and the prospective follow-up of the patients up to 96 weeks. Its main limitation is the lack of information on the liver histological pattern modification rather than indirect biomarkers, as it remains unclear whether their change truly reflects hepatic fibrosis change. The lack of information on patients' alcohol consumption and the absence of transient liver elastography measurements also represent limitations to this analysis.

Further studies are warranted to confirm an antifibrotic effect of CCR5 antagonist therapy.

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## PD-1+ and TIGIT+ CD4 T Cells Are Associated With Coronary Artery Calcium Progression in HIV-Infected Treated Adults

To the Editors:

With the advent of antiretroviral therapy (ART), AIDS-related morbidity

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and mortality has declined allowing age-related diseases, such as cardiovascular disease (CVD), to emerge as new challenges for this population. With an effect size of approximately 1.5–2.0, the impact of HIV on CVD is independent of traditional cardiovascular risk factors and antiretroviral medications.<sup>1</sup> Immune activation of monocytes and macrophages has been implicated in the higher CVD risk in individuals with chronic HIV. Coronary artery calcium (CAC) is an indicator of subclinical coronary artery atherosclerosis predictive of coronary events including the onset of myocardial infarction and coronary-related deaths.<sup>2</sup> Two research groups, including our own, have published that activated CD16<sup>+</sup> monocytes/macrophages predict greater CAC progression among HIV-infected persons over a 2-year period.<sup>3,4</sup>

HIV-associated immune dysfunction is also characterized by T-cell dysfunction; their role in HIV-associated atherosclerosis has been less studied. T-cell exhaustion is characterized by an expansion of negative checkpoint receptors (NCRs) including PD-1 (programmed cell death protein 1), TIM-3 (T-cell immunoglobulin and mucin-domain containing-3), and TIGIT (T-cell immunoreceptor with Ig and ITIM domains). During chronic infection or cancer, T-cell exhaustion results in the progressive loss of effector function, upregulation of inhibitory receptors, and failure to transition to a quiescent state.<sup>5</sup> We have recently reported that the expansion of NCR on CD4<sup>+</sup> T cells is associated with comorbidities of cognitive impairment and fat loss in HIV-infected individuals on ART.<sup>6,7</sup> In this study, we sought to examine the impact of NCR-expressing T cells on CVD. We hypothesized that higher baseline PD-1-expressing and/or TIGIT-expressing CD4<sup>+</sup> T cells will be associated with progression of CAC after 2 years in chronically HIV-infected individuals on stable ART.

T-cell immunophenotyping was performed on banked peripheral blood mononuclear cells from HIV-infected individuals enrolled in the Hawaii Aging with HIV-Cardiovascular Disease (HAHC-CVD) Cohort Study,<sup>8</sup> a longitudinal study of subclinical CVD risk in

individuals with chronic HIV age >40 years and on ART for ≥3 months. Data on CVD risk factors, as well as metabolic data from fasting blood, were available and allowed calculation of the Framingham Risk Score (FRS) using the National Cholesterol Education Program website (<http://hp2010.nhlbi.nih.net/atp/iii/calculator.asp>). As previously reported,<sup>8</sup> computer tomography (CT) examinations for CAC were performed locally in Honolulu, HI, using a dual-source CT scanner (Siemens 64-slice Somatom) with quantification of CAC centrally at the Los Angeles Biomedical Research Institute (M Budoff).

Cryopreserved peripheral blood mononuclear cell were thawed and stained for viability and the frequency of expression at baseline of TIGIT, PD-1, and TIM-3 on CD4<sup>+</sup> and CD8<sup>+</sup> T cells were assessed by flow cytometry following previously published methods.<sup>9</sup> Isotype controls or fluorescence minus one controls were used to facilitate gating. Software-based compensation was performed on FlowJo (Treestar).

The predictive impact of NCR-expressing T cells and other immunologic parameters on 2-year change in CAC was assessed by logistic regression, dichotomizing the participants into those who demonstrated progression of CAC and those whose level showed no progression. A multivariate logistic regression model was constructed. Because of the small sample size, FRS was used as a composite marker of traditional CVD-risk factors. We, as well as others, have reported a correlation between monocyte immune activation and change in CAC. We therefore further examined the relationship between NCR-expressing T cells and monocyte subsets based on CD16 expression (classical, intermediate, and nonclassical monocytes), hypothesizing that any impact of NCR-expressing T cells on CAC may be mediated through an increase in monocyte immune activation. A two-sided *P*-value (*P*) < 0.05 was considered as statistically significant. Analyses were performed using SPSS version 24 (IBM, Armonk, NY).

The data consisted of 43 HIV-infected participants who were predominantly male (88%) and Caucasian