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Short Communication

Patients on a combined antiretroviral therapy after maraviroc clinical test show no immunovirological impairment

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ABSTRACT

The maraviroc clinical test (MCT) is a clinical approach to establish the indication of maraviroc treatment. In this study, we analysed the long-term outcome of patients receiving a combined antiretroviral therapy (cART) selected according to MCT results. Ninety-two consecutive HIV-infected patients underwent MCT. A virological response (<40 HIV-RNA copies/ml after 24 weeks) was observed in 76/92 patients (82.6%). These patients (n = 76) were included in a time to treatment failure analysis; after a mean follow-up period of 88 weeks, treatment failure was confirmed in 14 patients (18.4%). Tropism switch during MCT was observed in 3/35 patients (8.6%); these patients experienced excellent long-term outcome on cART. In conclusion, MCT should be considered as an additional method before CCR5-antagonists prescription.

Determining viral tropism is mandatory before prescribing maraviroc (MVC). Trofile® and its new version (ES-Trofile®) are the assays that are predominantly performed in clinical practise (Whitcomb et al., 2007; Reeves et al., 2009). However, this test has some limitations, such as approximately 20% of non-reportable results and changes in the reported tropism results without therapeutic intervention (Schürmann et al., 2007; Landovitz et al., 2008). Different phenotypic (González et al., 2010) and genotypic assays (Raymond et al., 2008; Poveda et al., 2009; Chueca et al., 2009) have been developed as alternative tropism assays and compared with ES-Trofile®. Recently, we designed a clinical approach to establish the indication of CCR5-antagonist prescription (Maraviroc clinical test, MCT; Genebat et al., 2009). However, concerns about the potential emergence of CXCR4 (X4)-tropic virus during this short-term MVC exposure which could affect the virological efficacy of subsequent combined antiretroviral therapy (cART), have emerged. Thus, the objective of the present observational study was to analyse the long-term outcome of cART started after MCT.

Beginning on July 1st 2008, a prospective study was conducted in the Clinic Unit of Infectious Diseases, Microbiology and Preventive Medicine of Seville (Genebat et al., 2009). Briefly, asymptomatic HIV-infected patients with a persistently detectable viral load started eight-day MVC monotherapy (MCT). MCT was considered positive if a reduction >1 $\log_{10}$ HIV-RNA copies/mL or undetectable viral load (<40 HIV-RNA copies/mL) was achieved after eight-day MVC monotherapy. As of June 1st 2011, 92 consecutive HIV-infected patients underwent MCT. Subsequently, MCT patients began a new cART, according to (a) genotype resistance test, (b) previous antiretroviral exposure, and (c) response to MCT, to decide MVC inclusion in the new cART. Patients or guardians (for patients under 18 years old) provided written informed consent, and the Ethical Committee of the Hospital approved the study.

The virological response to cART was defined as a confirmed (two consecutive determinations) viral load <40 HIV-RNA copies/mL at week 24. Failure of the new cART was defined as the following: (a) persistently detectable viral load after at least 24 weeks of follow-up or (b) lost on follow-up (death or missing equals failure). Because patients included in the present study were both naive and pretreated subjects, we considered that an acceptable global rate of undetectability should be >75%, according to previous studies show-

Abbreviations: MVC, maraviroc; MCT, maraviroc clinical test; cART, CXCR4 (X4), combined antirretroviral therapy; D/M, dual/mixed.

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ing a global efficacy ranging from 68% (Recordon-Pinson et al., 2010) to 80% (Cooper et al., 2010). All of the patients achieving virological response were included in a time to treatment failure analysis (Kaplan–Meier). In this analysis, treatment failure was defined as a confirmed (two consecutive determinations) viral load >40 HIV-RNA copies/mL and lost on follow-up (death or missing equals failure). Additionally, the determination of HIV-1 co-receptor usage was performed using TROCAI, a phenotypic method developed in our laboratory (Ruiz-Mateos et al., 2004; González-Serna et al., 2010), in a subgroup of 35 consecutive patients the day of starting MCT and the last day of MCT with detectable viral load. An intention to treat analysis was performed in all cases.

Baseline characteristics of the patients are shown in Table 1. It should be noted that 14/92 patients (15.2%) showed detectable viral load <1000 HIV-RNA copies/mL (threshold of ES-Trofile®). MCT classified all of them as the following: 10 of these patients (71.4%) showed a positive MCT, while four (28.6%) showed no viral load modification after MCT. Patients with a positive MCT ($n = 67$) began an MVC-containing cART, while patients with a negative MCT ($n = 25$) began an MVC-sparing cART (summarised in Table 2). It should be noted that MVC was combined with low genetic barrier drugs (lamivudine/abacavir or raltegravir) or only atazanavir/ritonavir (a low potency drug in monotherapy) in 55 patients with a positive MCT (82.1%). Virological response was observed in 76 (82.6%) subjects and was greater in patients with a positive MCT (89.6% *vs* 64%; $p = 0.004$ chi-square test). Regarding the 16 patients with no virological response, 10 patients were lost on follow-up or non-adherent to cART. When patients on treatment were analysed separately, a virological response to cART after MCT was observed in 76/82 patients (92.7%). The proportion of patients with undetectable viral load at different time points is shown in Fig. 1. Mean $CD4^+$ T-cell gain after the cART was started is shown in Fig. 2.

TROCAI found tropism switch in 3/35 patients during MCT (8.6%). Two patients experienced a tropism change from R5 to dual/mixed (D/M); both of them had an undetectable viral load after 48 weeks of receiving an MVC-containing cART. Another patient with a negative MCT showed a tropism change from D/M to R5; this patient received an MVC-sparing cART and also achieved undetectability after 48 weeks.

Patients achieving virological response with the new cART ($n = 76$) were included in a Kaplan–Meier analysis to evaluate time to treatment failure (Fig. 3). After a mean follow-up of 88 weeks (95% CI 75-100), treatment failure was observed in 14/76 patients (18.4%). No significant differences were found regarding the proportion of treatment failures between MCT positive and MCT negative groups (log-rank test, $p = 0.18$).

These results show that MCT could be considered an additional approach to select candidate patients to receive CCR5-antagonists as part of a cART, because the majority of patients on treatment achieved a durable and favourable immunovirological response regardless of MVC utilisation. We have recently shown that response to MVC monotherapy is greater in patients with higher baseline $CD4^+$ T-cell counts (Ruiz-Mateos et al., 2011).

After MCT was designed (Genebat et al., 2009), the potential emergence of X4 variants that could affect further immunovirological evolution of the subsequent cART was hypothesised. In the present study, we did not observe the emergence of X4 variants during MVC monotherapy in >90% of patients. We have recently confirmed and extended these preliminary results (González-Serna et al., 2012a). The tropism switch from R5 to D/M could be explained by the presence of minor X4 variants before the initiation of treatment with a CCR5 antagonist (Fätkenheuer et al., 2005; Westby et al., 2006). The further evolution of these patients was satisfactory regarding the expected proportion of patients with a virological response, most likely because there is no clinical relevance of low-level X4 variants, as previously shown in the post hoc analysis of MERIT studies (Cooper et al., 2010). In contrast, the tropism switch from D/M to R5 could be explained by a categorical cut-off, suggesting that the clinical response to the drug may be more important than a categorical tropism result.

Different studies have shown that MVC use based on ES-Trofile® tropism prediction is safe over the long-term (Gulick et al., 2008; Nozza et al., 2011). Recently, we have shown similar results in a retrospective analysis in which MVC prescription was based on MCT results in most patients (Genebat et al., 2010). However, discordance rates of approximately 15% between MCT and ES-Trofile® (Genebat et al., 2011) have been recently described and might have important clinical implications. We consider that MCT could help to avoid ES-Trofile® limitations, such as the following: (1) changes in viral tropism reported by ES-Trofile® without any therapeutic intervention (Schürmann et al., 2007; Landovitz et al., 2008); (2) misclassification of patients as harbouring D/M tropism, as shown in the reanalysis of the MERIT study (Cooper et al., 2010); and (3) inability to analyse patients with low but detectable viral load (<1000 HIV-RNA copies/mL).

Genotypic methods are emerging in Europe as an alternative to ES-Trofile® (Raymond et al., 2008; Poveda et al., 2009; Chueca et al., 2009). When the MCT result was compared with different

Table 1
Baseline characteristics of the patients.

	Global ($n = 92$)	MCT positive ($n = 67$)	MCT negative ($n = 25$)	p value
Age, years	39.6 [8–70]	39.6 [8–70]	39.6 [15–63]	0.996
Male sex (%)	72 (78.3)	51 (76.1)	21 (84)	0.415
HCV coinfection[a] (%)	28 (30.4)	19 (28.4)	9 (36)	0.479
Viral load, $\log_{10}$ cop/mL	4.23 [1.94–6.03]	5.86 [1.94–4.17]	4.4 [2.08–6.03]	0.3
$CD4^+$, cell/mm^3	333.9 [2–913]	393.8 [18–913]	188 [2–646]	<0.001
Sexual transmission (%)	55 (59.8)	45 (67.2)	10 (40)	0.08
IDU[b] transmission (%)	30 (32.6)	19 (28.4)	11 (44)	0.1
Vertical transmission (%)	5 (5.4)	2 (3)	3 (12)	0.09
Blood transfussion (%)	2 (2.2)	1 (1.5)	1 (4)	0.3
Stage C, CDC[c] (%)	16 (17.4)	7 (10.4)	9 (36)	0.004
Real monotherapy[d] (%)	66 (71.7)	51 (76.1)	15 (60)	0.127
Functional monotherapy[e] (%)	26 (28.3)	16 (23.9)	10 (40)	0.127

Values other than percentage are expressed as mean [minimum–maximum]. Comparison of variables between groups was analysed using the Chi-square or Student's t test when stated.

[a] Positive PCR for hepatitis C virus.
[b] IDU: intravenous drug users.
[c] Centre for diseases control.
[d] Maraviroc monotherapy during MCT.
[e] Maraviroc added to the previous failing cART.

Table 2
Antiretrovirals started after MCT.

MCT negative patients (n = 25)MVC-sparing cART	n (%)	MCT positive patients (n = 67) MVC-containing cART[c]	n (%)
Darunavir/ritonavir plus raltegravir	12 (48)	Atazanavir/ritonavir	24 (35.8)
Two NRTIs plus NNRTI[a]	7 (28)	Lamivudine/abacavir	23 (34.3)
Two NRTIs plus IP[b]	6 (24)	Raltegravir	8 (11.9)
		Darunavir/ritonavir	6 (8.9)
		Darunavir/ritonavir plus etravirine	6 (8.9)

[a] cART based on two nucleosides (either abacavir/lamivudine or emtricitabine/tenofovir) plus one non-nucleoside (either nevirapine, efavirenz or etravirine).
[b] cART based on two nucleosides (either abacavir/lamivudine or emtricitabine/tenofovir) plus one protease inhibitor boosted with ritonavir.
[c] MVC combined with drugs exposed beneath.

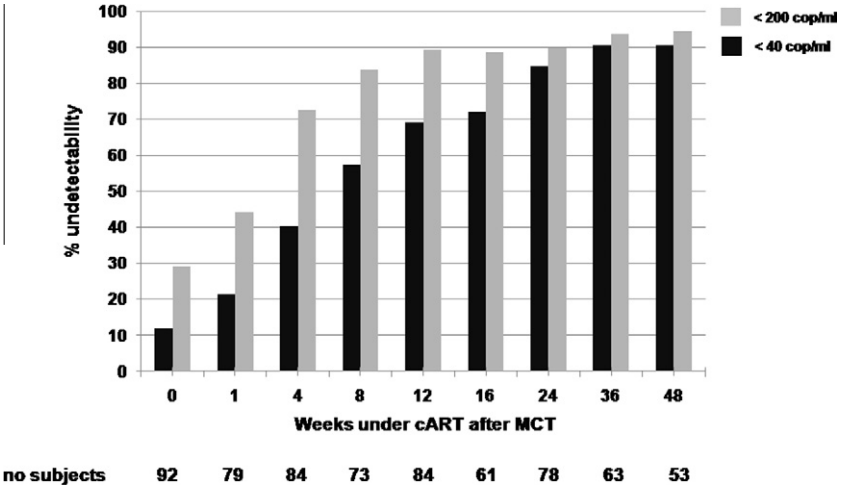

Fig. 1. Percentage of patients with undetectable viral load (black, <40 HIV–RNA copies/mL; grey, <200 HIV–RNA copies/mL) at each time point, after rescue therapy was started after MCT. After 48 weeks of follow-up (n = 53), >90% of patients had achieved undetectability. Note that 12% of patients started cART with an undetectable viral load; these patients achieved undetectability during MCT.

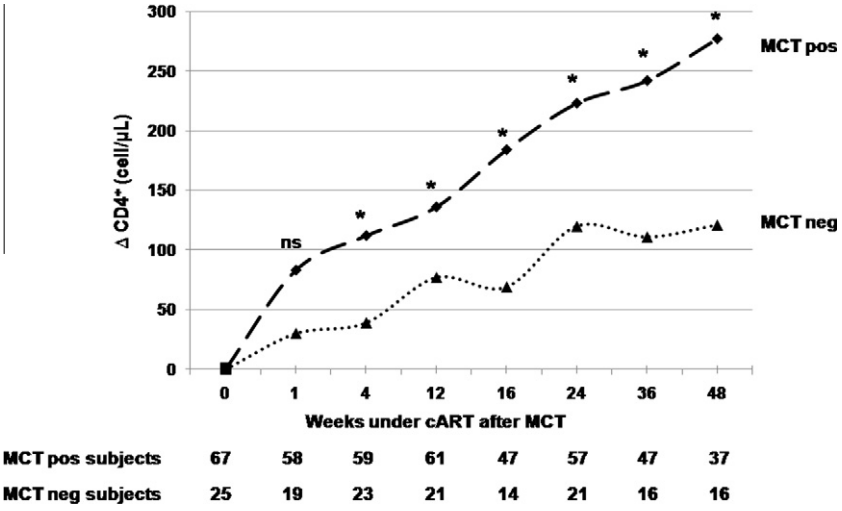

Fig. 2. Mean CD4$^+$ T-cell gain on cART after MCT according to MCT result: the MCT positive group showed a greater CD4$^+$ T-cell gain at every time point, except week one ($^*p < 0.05$, Mann–Whitney U test).

genotypic tests, including deep sequencing (González-Serna et al., 2012b), discordance rates of approximately 20% were observed. Moreover, genotypic methods have been compared with ES-Trofile® as the gold-standard, but the clinical decision to use MVC was established by ES-Trofile® instead of the genotypic tropism prediction. In fact, the present study is the first in which MVC clinical use in a prospective cohort was based on a different method and independent of ES-Trofile® results. Interestingly, in the present study, MVC was mainly combined with low genetic barrier drugs compared with previous studies in which background therapy was effective enough to achieve a virological response (Recordon-Pinson et al., 2010).

The main limitation of the present observational study is the absence of a control group. However, in our clinical practise,

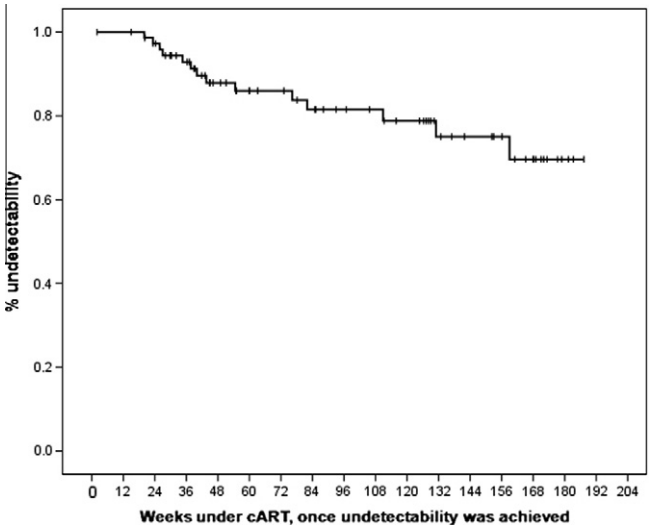

Fig. 3. Percentage of patients with undetectable viral load, after undetectability was achieved. Intention to treat analysis: confirmed detectable viral load, lost on follow up and death were considered as treatment failure.

MVC prescription is based on MCT due to the high rates of discordance of this method with other tropism assays (Genebat et al., 2011; González-Serna et al., 2012b).

In conclusion, based on the high rates of the virological success of a cART started after MCT, we believe that MCT should be considered as an additional method to be used in clinical practise before CCR5-antagonist prescription.

Conflict of interest

M.L. has a grant from ViiV Healthcare.

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