Switching the third drug of antiretroviral therapy to maraviroc in aviraemic subjects: a pilot, prospective, randomized clinical trial

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Objectives: To evaluate the safety and efficacy of switching the third drug of antiretroviral treatment to maraviroc in aviraemic subjects infected with R5 HIV.

Patients and methods: This is a pilot, prospective, randomized clinical trial (ClinicalTrials ID: NCT00966329). Eighty HIV-1-infected aviraemic adults on stable antiretroviral treatment for ≥1 year and no antiretroviral drug resistance were screened for the presence of non-R5 HIV by triplicate proviral V3 population sequencing. From them, 30 subjects with R5 HIV-1 were randomized 1:1 to switch the non-nucleoside reverse transcriptase inhibitor or ritonavir-boosted protease inhibitor to maraviroc (n = 15) or to continue the same antiretroviral treatment (controls, n = 15). The principal endpoint was the proportion of subjects with HIV-1 RNA <50 copies/mL at week 48. Ultrasensitive proviral HIV-1 tropism testing (454 sequencing) was performed retrospectively at weeks 0, 4, 12, 24, 36 and 48.

Results: One subject in the maraviroc arm and one control had non-R5 HIV in proviral DNA by retrospective 454 sequencing. The subject receiving maraviroc was the only individual to develop virological failure. However, plasma HIV at failure was R5. Switching to maraviroc was well tolerated and associated with small, but statistically significant, declines in total, high-density lipoprotein and low-density lipoprotein cholesterol. Median (IQR) triglyceride [1 (0.67–1.22) versus 1.6 (1.4–3.1) mmol/L, P = 0.003] and total cholesterol [4.3 (4.1–4.72) versus 5.4 (4.5–6.7) mmol/L, P = 0.059] values were lower in the maraviroc arm than in controls at week 48.

Conclusions: In this pilot, prospective, randomized clinical trial, switching the third drug to maraviroc was safe, efficacious and improved lipid parameters.

Keywords: ultrasensitive proviral HIV-1 tropism testing, virally suppressed patients, HIV

Introduction

It is uncertain if subjects with sustained undetectable HIV-1 RNA levels experiencing antiretroviral-related toxicity could safely switch their current ritonavir-boosted protease inhibitor (PI/r) or non-nucleoside reverse transcriptase inhibitor (NNRTI) drug to maraviroc. This treatment strategy could allow improvements in metabolic profile, efavirenz-related neurotoxicity, hepatotoxicity and gastrointestinal events, and would preserve other drugs with favourable toxicity profiles for future regimens. Nevertheless, for this strategy to be safe, it must be ensured that maraviroc is prescribed to subjects with negligible levels of CXCR4-using (non-R5) HIV.1 The safety of using population sequencing of the gp120 V3-loop of peripheral blood mononuclear cell (PBMC)-associated HIV-1 to guide maraviroc switches in aviraemic subjects, which is becoming increasingly popular in Europe,2,3 has been evaluated in observational studies,4,5 but not in prospective, randomized clinical trials.

We developed a pilot clinical trial to test the safety and efficacy of switching from a PI/r or an NNRTI to maraviroc in subjects with persistent virological suppression and with R5 viruses by proviral V3-loop population sequencing.
**Methods**

This was a 48 week, two-arm, prospective, randomized, pilot clinical trial in HIV-1-infected adults receiving care in the outpatient HIV Unit of the Hospital Universitari Germans Trias i Pujol, Badalona, Catalonia, Spain (ClinicalTrials ID: NCT00966329).

Inclusion criteria for screening were being on stable antiretroviral therapy including a PI/r or an NNRTI plus two reverse transcriptase inhibitors (NRTIs) for ≥1 year, having HIV-1 RNA <50 copies/mL for ≥6 months, absence of genotypic drug resistance to the NRTI backbone by bulk sequencing and ≥90% self-reported treatment adherence. Exclusion criteria were previous virological failure, previous detection of non-R5 HIV by any tropism test, active acute or uncontrolled neurological infection during the previous 2 months and pregnancy or willingness to become pregnant. The use of lipid-lowering drugs was allowed. The study was approved by the Institutional Review Board of the Hospital Universitari Germans Trias i Pujol. All subjects provided written informed consent to participate in the study.

HIV-1 tropism was inferred from subjects fulfilling the selection criteria from triplicate V3-loop population sequencing of proviral HIV-1 DNA (Supplementary methods, available as Supplementary data at JAC Online). Non-R5 HIV was defined as a false-positive rate value ≤DNA (Supplementary methods, available as Supplementary data at JAC Online). Non-R5 HIV was defined as a false-positive rate value ≤DNA (Supplementary methods, available as Supplementary data at JAC Online). Non-R5 HIV was defined as a false-positive rate value ≤DNA (Supplementary methods, available as Supplementary data at JAC Online). Non-R5 HIV was defined as a false-positive rate value ≤DNA (Supplementary methods, available as Supplementary data at JAC Online).

Eighty individuals were screened; 37 (46.2%) had non-R5 viruses by population sequencing, 13 did not fulfill the inclusion criteria and 30 entered the study, 15 per arm (Table 1). Subjects had been on antiretroviral treatment for a median of 7.4 (IQR 3.0–12.0) years and had had HIV-1 RNA <50 copies/mL for a median of 5.1 (IQR 2.7–8.8) years. Median (IQR) baseline and nadir CD4+ counts were 737 (515–850) and 336 (250–409) cells/mm³, respectively. All subjects allocated to the maraviroc arm were on NNRTI-based regimens at randomization: nine were receiving nevirapine and six were receiving efavirenz. Seven subjects allocated to the control arm were on PI/r-containing regimens (four on ritonavir-boosted atazanavir, two on ritonavir-boosted lopinavir and one on ritonavir-boosted fosamprenavir) and eight were on NNRTIs (five on efavirenz and three on nevirapine). The median (IQR) number of variants, reads and quality reads obtained by retrospective 454 sequencing per sample was, respectively: 299 (200–498), 3717 (1930–5850) and 2749 (1619–4282).

One patient in the control arm was lost to follow-up at week 12; one in the maraviroc arm interrupted therapy because of diarrhoea at week 1. There were no other adverse events.

One participant in the maraviroc arm and one control had non-R5 HIV-1 in the retrospective proviral DNA 454 sequencing analysis. See Figure 1(a and f). The subject receiving maraviroc

**Table 1.** Baseline characteristics of study participants

<table>
<thead>
<tr>
<th></th>
<th>Maraviroc arm</th>
<th>Control arm</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female gender, n (%)</td>
<td>1 (7)</td>
<td>1 (7)</td>
<td>0.759</td>
</tr>
<tr>
<td>Caucasian ethnicity, n (%)</td>
<td>15 (100)</td>
<td>15 (100)</td>
<td>1</td>
</tr>
<tr>
<td>Age (years)</td>
<td>42 (37–47)</td>
<td>39 (37–49)</td>
<td>0.412</td>
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<tr>
<td>Time since HIV diagnosis (months)</td>
<td>113 (37–187)</td>
<td>99 (63.5–156.7)</td>
<td>0.949</td>
</tr>
<tr>
<td>Time on suppressed viral load (years)</td>
<td>5 (2.8–9)</td>
<td>5.5 (2.2–8.8)</td>
<td>0.934</td>
</tr>
<tr>
<td>Time on highly active antiretroviral therapy (years)</td>
<td>7.1 (2.9–12.6)</td>
<td>7.7 (4.6–11.9)</td>
<td>0.836</td>
</tr>
<tr>
<td>Baseline CD4+ T cell count (cells/mm³)</td>
<td>639 (430–770)</td>
<td>791 (542–996)</td>
<td>0.098</td>
</tr>
<tr>
<td>Nadir CD4+ T cell count (cells/mm³)</td>
<td>319 (212–373)</td>
<td>342 (263–424)</td>
<td>0.436</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>5 (4.8–5.2)</td>
<td>4.8 (3.8–5.6)</td>
<td>0.744</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.3 (1.15–1.5)</td>
<td>1.16 (0.96–1.4)</td>
<td>0.126</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>2.9 (2.7–3.2)</td>
<td>3.1 (1.9–3.4)</td>
<td>1</td>
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<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.2 (0.8–1.7)</td>
<td>1.7 (0.9–2.5)</td>
<td>0.202</td>
</tr>
<tr>
<td>Glycaemia (mmol/L)</td>
<td>5.3 (5.1–5.5)</td>
<td>4.9 (4.7–5.2)</td>
<td>0.005</td>
</tr>
</tbody>
</table>

Values are shown as median (IQR), unless otherwise indicated.
Figure 1. Study outcomes. The main results of the study are shown, i.e.: virological efficacy according to the ‘per-protocol’ or ‘intention-to-treat, where switching or missing equalled failure, analyses (a); median and IQR CD4+ counts during the study (b); median lipid values at baseline and week 48 (c); and proportion of subjects with dyslipidaemia, according to the National Cholesterol Education Programme (NCEP, Adult Treatment Panel III) borderline-high cut-offs [i.e. triglycerides, ≥1.7 mmol/L (≥150 mg/dL); total cholesterol, ≥5.2 mmol/L (≥200 mg/dL); HDL cholesterol, <1.03 mmol/L (<40 mg/dL); and LDL cholesterol, ≥3.4 mmol/L (≥130 mg/dL)] (d). (e and f) Frequency of non-R5 HIV detected in each individual allocated to the maraviroc and control arms, respectively. Different symbols correspond to different subjects. Values corresponding to 0% frequency are not depicted because of the logarithmic scale of this representation. The grey area highlights levels of non-R5 viruses present below the pre-specified 2% detection limit. In all panels, P values <0.1 are shown. MVC, maraviroc.
was the only individual developing virological failure during the study (HIV RNA = 180 and 170 copies/mL at week 36). This subject had switched from nevirapine to maraviroc on a tenofovir/emtricitabine backbone. Trough drug plasma concentrations 12 h 30 min post-dose were 454 ng/mL for maraviroc (18.2 x the target), 78 ng/mL for tenofovir (1.2 x the Cmin) and 179 ng/mL for emtricitabine (2 x the Cmin) at the time of virological failure. By bulk sequencing, the rebounding virus was R5 (Geno2Pheno [coreceptor] false-positive rate: 52.8%) and had no resistance mutations in protease or reverse transcriptase. The subject regained HIV-1 RNA suppression <50 copies/mL after switching back from maraviroc to nevirapine. All remaining subjects retained HIV-1 RNA <50 copies/mL throughout the study.

Therefore, in the primary efficacy analysis by intention-to-treat, where switching or missing equalled failure, 13 individuals (86%) in the maraviroc arm and 14 (93%) in the control arm achieved HIV-1 RNA <50 copies/mL at week 48. In the per-protocol analysis, only one subject in the maraviroc arm developed virological failure (Figure 1a).

Median CD4⁺ cell counts remained >500 cells/mm³ in both arms (Figure 1b). There was a median (IQR) increase of 75.5 (−130 to 190) cells/mm³ in the maraviroc arm (P = 0.397) and a median decrease of 84 (−153.5 to −22.25) cells/mm³ in the control arm (P = 0.035), relative to baseline values. At week 48, differences between groups were not significant (P = 0.085).

Four subjects in the maraviroc arm and three controls were on lipid-lowering drugs at baseline; one subject in the control arm began lipid-lowering treatment at week 12; they all continued receiving these drugs until the end of the study. Median lipid values were not significantly different at baseline and remained within the normality range throughout the study in both arms. Subjects in the maraviroc arm showed small, but statistically significant, declines in total cholesterol [from 5 (4.8–5.2) to 4.3 (4.1–4.72) mmol/L, P = 0.003], HDL cholesterol [from 1.3 (1.15–1.52) to 1.25 (1.08–1.52) mmol/L, P = 0.004] and LDL cholesterol [from 2.9 (2.7–3.2) to 2.5 (2.37–2.66) mmol/L, P = 0.005] between baseline and week 48. There were no significant longitudinal changes in median lipid values in the control group.

At week 48, median (IQR) triglyceride values were significantly lower in the maraviroc arm [1 (0.67–1.22) mmol/L] than in controls [1.6 (1.4–3.1) mmol/L] (P = 0.003). Median (IQR) total cholesterol values were also lower in the maraviroc arm [4.3 (4.1–4.72) mmol/L] than in the control arm [5.4 (4.5–7.5) mmol/L], but only achieved marginal statistical significance (P = 0.059) (Figure 1c).

Nine subjects (60%) in each study arm had abnormal lipid values at baseline (Figure 1d). Abnormal triglyceride, total cholesterol, HDL cholesterol and LDL cholesterol values were found in nine versus five, seven versus nine, five versus two and eight versus six in controls and maraviroc switches, respectively. Fewer subjects in the maraviroc arm had hypercholesterolaemia than in the control arm (21.4% versus 64.3%, respectively, P = 0.054) at week 48. There were no statistically significant differences between arms in the proportion of subjects with other types of dyslipidaemia (Figure 1d). The total cholesterol/HDL cholesterol atherogenic ratio decreased in the maraviroc arm (from 3.78 to 3.31), but increased in the control group (from 3.93 to 4.44) between baseline and week 48; however, differences between and within groups did not achieve statistical significance.

Median (IQR) glycaemia values remained within the normality range without significant differences between arms.

Discussion

In this pilot, prospective, randomized clinical trial, switching the third drug to maraviroc was safe, efficacious, well tolerated and improved lipid parameters.

The virological efficacy of maraviroc in our study supports genotypic tropism testing of proviral DNA as a suitable tool to guide treatment switches to CCR5 antagonists in aviraemic individuals. If the test had been unable to screen out subjects with significant levels of non-R5 HIV, they would have been exposed to virtual dual-NRTI therapy for 48 weeks. Conceivably, that would have been associated with non-R5 virus evolution, higher rates of virological failure and potential development of NRTI resistance. Importantly, no subject with R5 HIV at baseline developed non-R5 viruses during continued exposure to maraviroc therapy, suggesting that maraviroc-including regimens continued to inhibit viral replication as much as conventional antiretroviral treatment.

The settings used for proviral DNA population sequencing in this study were more conservative than the ones suggested by the European tropism guidelines, but still missed non-R5 HIV in the only subject that eventually developed virological failure. The clinical implications of this finding are uncertain, however, as the rebounding plasma virus was R5 and had no resistance mutations in protease or reverse transcriptase. Trough drug levels at the time of viraemia rebound were adequate, which rules out major adherence or pharmacokinetic problems as the cause of virological failure. Eventually, the subject regained virological suppression when he was switched back to tenofovir/emtricitabine plus nevirapine. Non-R5 levels remained relatively stable in proviral DNA throughout the study follow-up, even at virological failure. This is concordant with the heterogeneous cellular and viral composition of PBMCs, which often includes non-viable or non-replicating viruses, although we cannot rule out the emergence of mutations conferring resistance to maraviroc, as observed in previous studies. Larger studies are needed to clarify whether there is a relationship between levels of non-R5 HIV in PBMCs and virological outcomes to maraviroc therapy in aviraemic subjects, beyond the sensitivity threshold of ultrasensitive genotypic tropism techniques.

As observed in treatment-naïve individuals, a switch to maraviroc improved lipid parameters, with small, but statistically significant, reductions in median total cholesterol and LDL cholesterol levels, and lower triglyceride and total cholesterol levels relative to controls at the end of the study. This lipid-lowering effect, alongside the putative anti-inflammatory activity of maraviroc, could potentially contribute to reduce antiretroviral therapy-related cardiovascular risk, allowing some patients to avoid lipid-lowering agents and their associated toxicity or drug interactions with antiretrovirals. Indeed, we observed a non-significant trend towards decreasing total cholesterol/HDL cholesterol ratios in subjects switching to maraviroc, relative to controls that should be confirmed in larger studies. Although the study inclusion criteria did not restrict for previous
NNRTI or PI use and patients were not stratified according to previous treatment, all subjects who switched to maraviroc in this trial were previously receiving NNRTIs. This could have influenced the lipid results even though lipid levels were not significantly different between the study arms at baseline. Particularly, improvements in the lipid profile might have been more evident in subjects switching to maraviroc from PI/r-containing regimens.

The main limitation of this study was its small sample size, which is justified by its pilot nature, given the risk of this strategy and the lack of prospective, randomized, clinical trials assessing its safety and virological consequences when the study was designed. Although our results cannot be considered definitive, they are highly informative for upcoming, well-powered clinical trials. On the one hand, clinicians should be reassured of the overall good performance of genotypic tropism testing in proviral DNA as a tool to screen for aviraemic subjects suitable to receive CCR5 antagonists. On the other hand, studies should attempt to confirm whether ultrasensitive genotyping techniques could further improve clinical outcomes. Cost-effectiveness analyses as well as more detailed metabolic studies would also be important to define the best clinical use of this treatment strategy. One final limitation, common to all clinical trials, is that the benefits of the approach tested in this study could have been overestimated in comparison with routine clinical practice, as participants in clinical trials usually have higher treatment adherence and motivation to pursue treatments correctly.

In conclusion, this pilot, prospective, randomized clinical trial showed that switching the third drug of antiretroviral therapy to maraviroc in aviraemic subjects is safe, efficacious and improves lipid parameters. Adequately powered studies should corroborate our findings, evaluate the cost-effectiveness of this treatment approach and, in particular, investigate whether detection of pre-existing low-frequency non-R5 HIV-1 in proviral DNA further improves the efficacy of this strategy.

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Supplementary data
Supplementary methods are available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

References

