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FULL PAPER

48 week outcomes of maraviroc-containing regimens following the genotypic or Trofile assay in HIV-1 failing subjects: the OSCAR Study

Silvia Nozza[1], Angela Rosa Pignataro[2], Laura Galli[1], Valentina Svicher[4], Claudia Alteri[4], Enzo Boeri[2], Marco Ripa[1,3], Antonella Castagna[1,3], Michela Sampaolo[2], Massimo Clementi[2,3], Carlo Federico Perno[4], Adriano Lazzarin[1,3]

[1]*Department of Infectious Diseases, IRCCS San Raffaele, Milan, Italy;*
[2]*Laboratory of Microbiology and Virology, IRCCS San Raffaele, Milan, Italy;*
[3]*Università Vita-Salute San Raffaele, Milan, Italy;*
[4]*Department of Experimental Medicine and Surgery, University of Rome Tor Vergata, Italy*

SUMMARY

This study assessed the 48-week efficacy of an antiretroviral therapy including maraviroc following the assessment of co-receptor tropism by use of Geno2Pheno algorithm or the Trofile phenotypic assay in failing treatment-experienced HIV-1 patients.
This was a multicenter, randomized, open-label, non-inferiority trial. Treatment-experienced subjects with HIV-RNA ≥500 copies/mL were randomized (1:1) to undergo co-receptor tropism testing by the Geno-2Pheno algorithm (with a false positive rate >10%) or the Trofile assay before starting a new antiretroviral treatment which included maraviroc. The primary endpoint was the 48 week proportion of patients with treatment success (TS). Intention-to-treat analyses are also reported.
One hundred and fifty-five experienced patients were analysed: 77 patients in the Trofile arm and 78 in the Genotype arm. The 48-week proportion of TS was 87% in the Trofile arm and 89% in the Genotype arm (difference: 1.5%, 95%CI: -8.9% to 11.8%) suggesting non-inferiority. In the Trofile arm, 10 patients had treatment failure: 5 viral rebound, 5 discontinuations. In the Genotype arm, 9 patients had treatment failure: 7 viral rebound, 2 lost to follow-up. CD4+ significantly increased from baseline to week 48 in both arms.
48-week treatment success was similar for maraviroc-including therapy prescribed following the Trofile phenotypic assay or Geno2Pheno algorithm.

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INTRODUCTION

HIV-1 enters and infects target cells using the cellular CD4 receptor and coreceptor CCR5 and CXCR4 (Berger EA *et al.,* 1999). CCR5 antagonists inhibit HIV entry by altering the conformation of CCR5 at the cell surface and disrupting interaction with gp120 (Esté *et al.,* 2007). During the registration trials of maraviroc (Gulick *et al.,* 2008), the recombinant phenotypic Trofile assay (Monogram Biosciences) (Withcomb *et al.,* 2007) was the most frequently used co-receptor tropism test. Nevertheless, the genotypic tropism test is a practical alternative method that sequences the V3 loop of HIV gp120 allowing tropism inference using bioinformatics algorithms (Sing *et al.,* 2007).
The MOTIVATE-1 and 2 studies suggested that in treatment-experienced individuals Geno2Pheno is a potential useful tool for identifying subjects who could benefit from maraviroc treatment (McGovern *et al.,* 2010).

Key words:
Maraviroc, Failing patients, Trofile, Genotypic tropism assay.

Corresponding author:
Silvia Nozza
E-mail: nozza.silvia@hsr.it

There are no data on the application of Geno2Pheno in prospective studies.
The OSCAR study was set up to compare the performances of genotypic HIV-1 tropism testing in comparison to the Trofile assay (virological study) and to assess the virological efficacy of an antiretroviral therapy including maraviroc (clinical study).
The OSCAR virological study (Svicher *et al.,* 2010) evaluated 406 patients (255 treatment-experienced) with HIV-RNA ≥500 copies/mL. Both Trofile and Geno2Pheno were performed and the results showed that genotypic-tropism testing at a False Positive Rate (FPR) of 10% was 78.4% concordant with Trofile (reference assay) (Vandekerckhove *et al.,* 2011).
Now the aim is to report the virological efficacy of the OSCAR clinical study in failing treatment-experienced patients who started a maraviroc (MVC)-including therapy after co-receptor tropism assessment by use of Genotype or the Trofile.

MATERIALS AND METHODS

Study population

The OSCAR Study (EUDRACT 2010-018709-12) is a multicenter, non-inferiority, randomised, open-label, parallel group trial on adult treatment-experienced HIV-infected

subjects failing HAART with HIV-RNA ≥500 copies/mL. All patients signed informed consent approved by the local Ethical Committees.

Patients were experienced to three drug classes: NRTIs, NNRTIs and PIs and had to have an active drug in the regimen. Eligible patients were randomised 1:1 to assess co-receptor tropism by the Geno2Pheno or the Trofile (screening). Only patients with an R5-tropic virus were enrolled and started treatment with maraviroc + optimized background therapy. Maraviroc dosage was prescribed according to the other therapy. Enrolled subjects were followed-up at weeks 4, 8, 12, 24, 36 and 48. At baseline, an additional blood sample was collected to allow Geno2Pheno co-receptor assessment in patients randomized to the Trofile assay and vice versa in patients randomized to the Trofile assay. The result of this additional evaluation was stored at the principal investigator's site and was blind for the patient's physician.

The primary objective of the study was to compare the 48-week treatment success and the primary endpoint was the proportion of patients with HIV-RNA<50 copies/mL at week 48. The secondary objectives were the assessment of the time to treatment failure and the immunological (CD4, CD4%, CD8, CD8%) changes at week 48.

Viral tropism determination

Tropism was determined on RNA plasma samples by Trofile test or by V3-loop sequencing interpreted using the Geno2Pheno algorithm. A single PCR product per sample was subjected to standard population sequencing. Sequences were analysed with Seqscape software v2.5 (Applied Biosystems, Foster City, CA, USA). Nucleotide mixtures were considered if the second highest peak in the electropherogram was >25%. Co-receptor tropism was inferred with the Geno2Pheno algorithm. Clonal prediction was employed for classifying sequences; viral isolates were classified as R5 if FPR >10%.

Statistical analysis

A target sample size of 244 patients (122 per arm) provided 80% power (one-sided, alpha 0.05) to establish the non-inferiority of the Genotype arm compared to the Trofile arm with an overall treatment success rate of 85% at week 48. However, the target sample size was not completed due to the limited effectiveness of the recruitment phase which was stopped 20 months after the start of the study. To assess non-inferiority, the difference between the proportions of treatment success in the two arms (Geno2Pheno - Trofile) was calculated together with the corresponding 95% confidence interval. Non-inferiority was verified if the lower limit of the 95% confidence interval did not exceed the pre-specified non-inferiority margin of -10%.

The primary analysis on the primary endpoint was performed on the intention-to-treat (ITT) population consisting of all the randomized subjects treated with at least one dose of the study treatment. Patients lost to follow-up or who stopped/modified the assigned treatment for virological failure or any other cause were considered failures (treatment failure).

Secondary efficacy analyses on the primary endpoint were also performed on the on-treatment population (OT) including all patients from the ITT population except those who withdrew from the study or discontinued the study for any reason. The analyses on the secondary endpoints were performed on the ITT population.

Results were described as median (interquartile range, IQR) or frequency (%).

Patients' characteristics were compared by the Mann-Whitney test or the chi-square/Fisher exact test, as appropriate; 48 week changes from baseline were tested by the Wilcoxon signed rank test. A two-sided alpha level of 0.05 was taken as reference to detect statistical significance in all analyses. The statistical analyses were performed using the SAS Software, release 9.2.

RESULTS

Between July 2010 and February 2012, 155 patients were randomized and underwent tropism evaluation before starting a new antiretroviral treatment which included maraviroc (ITT population): 77 patients assessed tropism by the Trofile test and 78 patients by the Geno2Pheno algorithm. Baseline characteristics were well balanced between the two groups (*Table 1*).

In the Trofile arm, 5 patients (6.5%) had a false-positive rate <10% compared to the Geno2Pheno algorithm. No discordant results were observed between the two tropism tests in the Genotype arm. The median value of FPR was 41% (25%-56%) in the Genotype arm and 34% (27%-63%) in Trofile arm.

At the primary efficacy analysis, the proportion of treatment success was 87% in the Trofile arm and 89% in the Genotype arm (*Figure 1*; difference: 1.5%, 95%CI: -8.9% to 11.8%). The analysis stratified according to HIV-RNA at the start of the MVC-including treatment showed an unfavourable difference in efficacy for patients with high levels of HIV-RNA (*Figure 1*).

In the Trofile arm, 10 patients had treatment failure by week 48: 5 patients had confirmed viral rebound and 5 patients discontinued the study [1 grade 3 hepatic toxicity, 2 patient's decision, 1 lost to follow-up, 1 death due to non Hodgkin's lymphoma].

In the Genotype arm, 9 patients had treatment failure by week 48: 7 patients had confirmed viral rebound and 2 patients were lost to follow-up. Time to treatment failure was also similar between the two groups (log-rank test: p=0.758; *Figure 2*).

Secondary endpoints

The CD4+, CD4% and CD4+/CD8+ ratio significantly increased during follow-up with no significant differences between the two arms.

The 48-week change in CD4+ was 128 (60-259) cells/mmc in the Trofile arm (p<0.0001) and 161 (43-260) cells/mmc in the Genotype arm (p<0.0001) [Trofile vs Genotype: p=0.668]; CD4% change was 3.9% (0.6%-7.0%) in Trofile arm (p<0.0001), 3.4% (1.0%-6.9%) in Genotype arm (p<0.0001) [Trofile vs Genotype: p=0.447]; CD4/CD8 ratio change was 0.14 (0.05-0.24) in Trofile arm (p<0.0001), 0.14 (0.05-0.22) in Genotype arm (p<0.0001) [Trofile vs Genotype: p=0.693].

Similarly, CD8+ cell counts (*Figure 3*) and CD8% significantly decreased during follow-up and no significant differences were detected between the two arms: the 48-week change was 19 (-269/+169) cells/mmc in Trofile arm (p<0.0001), -41 (-221/+160) cells/mmc in the Genotype arm (p<0.0001) [Trofile vs Genotype: p=0.683]; 48-week change in CD8%: -6.6% (-10.1%/-2.6%) in Trofile arm (p<0.0001), -5.6% (-10.9%/-1.4%) in the Genotype arm (p<0.0001) [Trofile vs Genotype: p=0.672].

Table 1 - *Baseline characteristics of the 155 HIV-1 infected patients of the OSCAR clinical study.*

	Trofile (N=77)	*Geno2Pheno (N=78)*	*P-value*
Age, *years*	46.0 (43.1-49.7)	45.0 (41.1-49.7)	0.416[a]
Males	61 (79%)	66 (85%)	0.411[b]
Years of HIV infection	16.2 (12.7-20.9)	13.8 (10.9-18.8)	0.034[a]
HIV risk factor			0.077[b]
IDU	15 (19%)	12 (15%)	
MSM	28 (36%)	41 (53%)	
Heterosexual	23 (30%)	22 (28%)	
Other/unknown	11 (14%)	3 (4%)	
CDC C Stage	19 (25%)	21 (27%)	0.855[a]
ART duration, years	12.8 (10.5-15.5)	12.0 (7.4-15.1)	0.231[a]
CD4+ nadir, *cells/μL*	174 (54-287)	176 (74-274)	0.692[a]
CD4+, *cells/μL*	330 (214-473)	386 (236-588)	0.135[a]
CD4%	17.8 (12.2-23.6)	18.9 (11.1-26.5)	0.686[a]
CD8+, *cells/μL*	909 (702-1208)	1112 (890-1445)	0.065[a]
CD8%	53.2 (47.9-59.3)	51.7 (45.4-62.9)	0.833[a]
CD4+/CD8+ ratio	0.34 (0.18-0.50)	0.32 (0.18-0.56)	0.945[a]
HIV-RNA, *log_{10} copies/mL*	4.2 (3.5-4.8)	3.9 (3.4-4.5)	0.204[a]
Previous antiretroviral regimen			
NRTIs-including regimens	10 (18%)	14 (23%)	
NNRTIs-including regimens	7 (13%)	6 (10%)	0.777
PIs-including regimens	32 (57%)	36 (59%)	
Raltegravir-including regimens	7 (13%)	5 (8%)	
Baseline antiretroviral regimen			
MVC and NRTIs-including regimens	10 (13%)	11 (14%)	
MVC and NNRTIs-including regimens	1 (1%)	1 (1%)	0.897
MVC and PIs-including regimens	19 (25%)	23 (30%)	
MVC and raltegravir-including regimens	47 (61%)	43 (55%)	

Abbreviations: IDU, intravenous drug user; MSM, men who have sex with men; ART, antiretroviral treatment; MVC, maraviroc.
Results as median (IQR) or frequency (%).
[a]by Wilcoxon rank sum test.
[b]by chi-square or Fisher exact test, as appropriate.

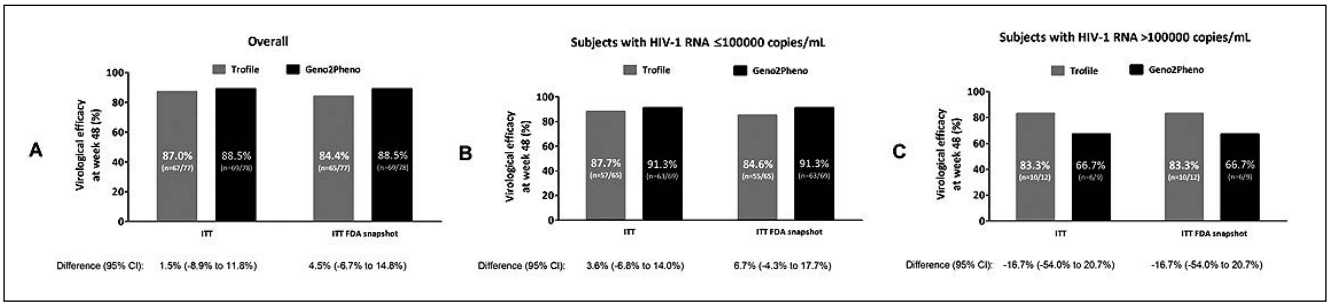

Figure 1 - *Virological efficacy according to tropism assay, ITT and OT analysis. All patients (panel A), patients with baseline HIVRNA ≤100.000 copies/mL (panel B) and patients with baseline HIVRNA>100.000 copies/mL (panel C).*

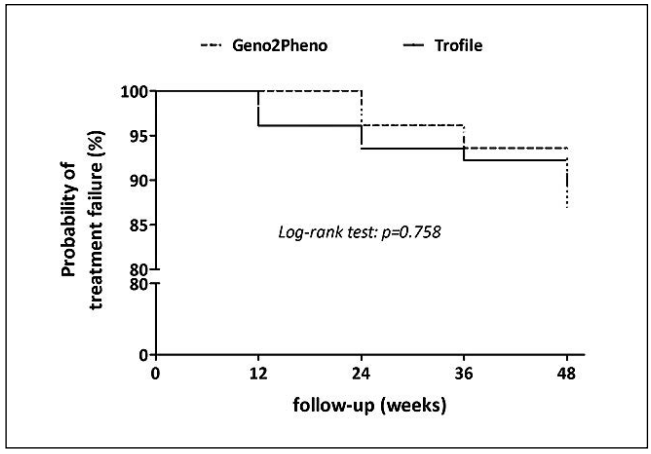

Figure 2 - *Probability of treatment failure according to tropism assay.*

pendently associated with virological response to MVC (Svicher *et al.,* 2013; Recordon-Pinson *et al.,* 2010). CD4+ cells count significantly increased in both arms; we previously found a greater immune recovery with maraviroc containing ART, due to a reduction of activated T cells (Cossarini *et al.,* 2012).

We acknowledge that the small number of subjects is a major limitation with a clear impact on the statistical power and, thus, on the strength of our results; nevertheless, efficacy rates were very similar.

In conclusion, the use of Geno2Pheno algorithm to select patients to treat with a MVC-including regimen led to similar treatment success as the use of Trofile assay, supporting the use of this algorithm in clinical practice, as in some other countries (http://www.salute.gov.it/portale/documentazione/p6_2_2_1.jsp?lingua=italiano&id=2442 http://www.gesida-seimc.org/contenidos/guiasclinicas/2015/gesida-guiasclinicas-2015-tar.pdf).

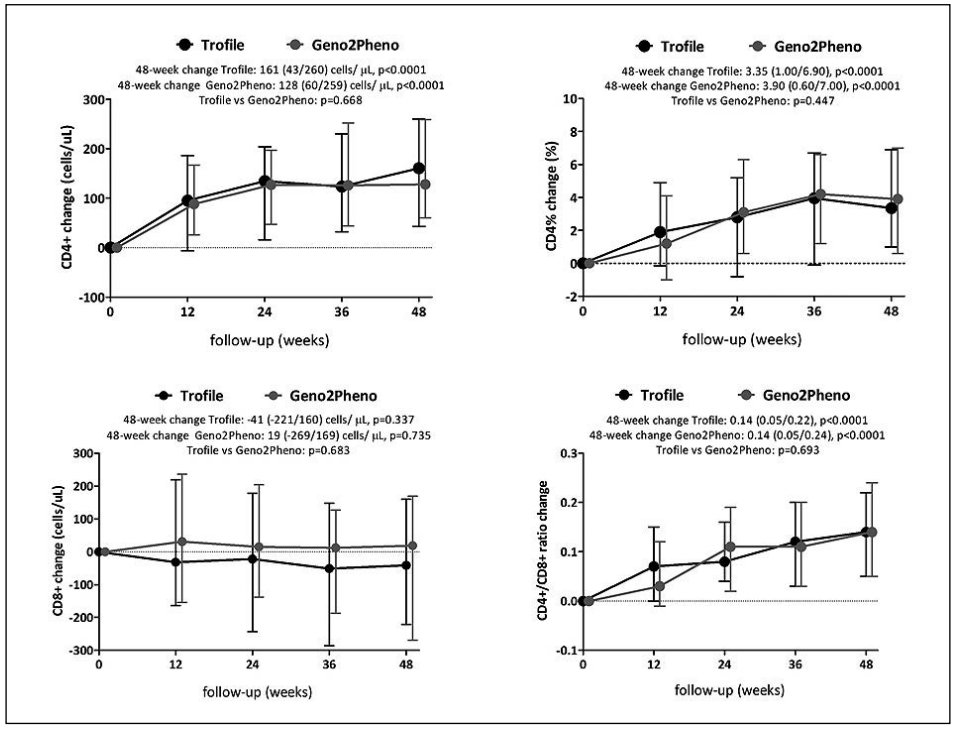

Figure 3 - *Immunological response according to tropism assay.*

DISCUSSION

The OSCAR clinical study is the first prospective trial comparing efficacy in patients treated with a maraviroc-including regimen after assessment of co-receptor tropism with the Trofile phenotypic assay or Geno2Pheno algorithm.

The study showed no difference between arms with respect to the rate of treatment success at week 48. This finding seems to be in line with previous retrospective studies (McGovern *et al.,* 2012; Frange *et al.,* 2009) demonstrating the efficacy of the genotypic test to predict virological response. In this study we also found a lower efficacy in patients with high viral load (>100000 copies/mL) at the start of the maraviroc-including regimen. This finding has already been reported in a previous study which found that a lower baseline HIV-RNA (as well as a GSS≥1 and a higher nadir CD4) was inde-

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