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Virological and Immunological Response to Antiretroviral Regimens Containing Maraviroc in HIV Type 1-Infected Patients in Clinical Practice: Role of Different Tropism Testing Results and of Concomitant Treatments

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Abstract

We assessed the immunovirological response to antiretroviral regimens containing maraviroc in HIV-infected viremic patients with viral tropism predicted by different assays. We selected antiretroviral treatment-experienced HIV-1-infected patients initiating regimens containing maraviroc after different phenotypic or genotypic viral tropism assays, with at least one HIV-1 RNA determination during follow-up. Survival analysis was employed to assess the virological response as time to HIV-1 RNA <50 copies/ml and immunological response as time to a CD4 cell count increase of $\geq 100/\mu$l from baseline. Predictors of these outcomes were analyzed by multivariate Cox regression models. In 191 treatments with maraviroc, virological response was achieved in 65.4% and the response was modestly influenced by the baseline viral load and concomitant drug activity but not influenced by the type of tropism assay employed. Immunological response was achieved in 58.1%; independent predictors were baseline HIV-1 RNA (per $\log_{10}$ higher: HR 1.29, 95% CI 1.05–1.60) and concomitant therapy with enfuvirtide (HR 2.05, 0.96–4.39) but not tropism assay results. Of 17 patients with baseline R5-tropic virus and available tropism results while viremic during follow-up on maraviroc, seven (41%) showed a tropism switch to non-R5 virus. A significant proportion of experienced patients treated with regimens containing maraviroc achieved virological response. The tropism test type used was not associated with immunovirological response and concomitant treatment with enfuvirtide increased the chance of immunological response. More than half of virological failures with maraviroc were not accompanied by tropism switch.

Introduction

Six different classes of antiretroviral drugs have been developed that target HIV-1 replication at different stages. Among these, chemokine receptor 5 (CCR5) antagonists selectively inhibit the entry into host cells of CCR5-using (R5) HIV-1 strains by an allosteric mechanism after binding to the transmembrane CCR5 coreceptor cavity.[1–6]

Current European guidelines indicate a mandatory coreceptor tropism test in all cases in which a CCR5 antagonist is

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being considered as part of the subsequent regimen, such as virological failure, the need to change a successful regimen because of toxicity or inconvenience, and treatment of drug-naive patients in whom toxicity to common first-line treatments is expected.[7]

Several methodologies for determining HIV-1 coreceptor tropism are available, including genotypic and phenotypic approaches, but actually there is no diagnostic gold standard.[7,8] Among the phenotypic tropism tests, the original Trofile assay (Monogram Biosciences, San Francisco, CA) allowed detection of CXCR4-using (X4) strains at a prevalence of ≥10% of the viral quasispecies. Since June 2008, a more sensitive assay version, the "enhanced sensitivity Trofile assay" (ESTA), has been introduced in clinical routine. This version increased the detection limit of minority X4 strains down to 0.3%.[9–11] On the other hand, genotypic tropism testing is based on amplification and sequencing of the gp120 V3 loop region and its interpretation using several bioinformatic algorithms such as the position-specific scoring matrix (PSSM) and the geno2pheno$_{[coreceptor]}$ (G2P) system.[12]

Few observational studies have previously investigated the virological and immunological response to antiretroviral treatment (ART) regimens containing maraviroc in HIV-1-infected patients and their association with patient-related and virologic variables.[13,14]

Here we present the immunovirological outcome of patients undergoing maraviroc-based treatment in clinical practice where coreceptor tropism was determined by different assays. Correlates of immunological and virological responses were also analyzed and are presented here.

Materials and Methods

Study design and patients

We retrospectively examined HIV-1-infected patients initiating maraviroc-containing ART regimens between July 2005 and April 2011. These were all treatment-experienced patients, enrolled in the Antiretroviral Resistance Cohort Analysis (ARCA), a national observational cohort of HIV-1-infected patients followed by >100 clinical and laboratory units in Italy (www.hivarca.net). All patients were anonymous and were included in the ARCA database after signing an informed consent to provide their data for academic not-for-profit studies. The ARCA initiative is compliant with the Declaration of Helsinki and each participating center is subject to a local Ethics Committee that follows national (and where applicable European) regulations. Additional inclusion criteria for the study were availability of plasma HIV-1 RNA load within 120 days prior to maraviroc treatment initiation and of at least one HIV-1 RNA determined subsequent to maraviroc treatment initiation.

The following variables were retrieved for all enrolled patients from the information available in the ARCA database, using the date of maraviroc treatment initiation as the baseline time point: calendar year, age, gender, nation of birth, viral subtype, time since HIV diagnosis, baseline HIV-1 RNA, baseline CD4$^+$ T cell count, nadir CD4$^+$ T cell count, mode of HIV-1 transmission, time from the first HIV-1-positive antibody test to the first ART initiation, duration of prior antiretroviral exposure, number of previous antiretroviral drugs employed, and number of previous antiretroviral treatment lines employed.

Determination of viral tropism and calculation of the genotypic susceptibility to drugs accompanying maraviroc

All patients underwent testing for HIV-1 coreceptor tropism with the use of at least one of the following assays: a phenotypic assay, namely Trofile or ESTA, or a genotypic assay. Some patients underwent a combined viral tropism assessment using both a genotypic and a phenotypic assay. Genotypic analysis of the nucleotide sequence of *env* coding for the gp120 V3 region was performed in a single assay by population sequencing and results were interpreted using the clonal G2P prediction algorithm; the reported false positive rate (FPR) was used as quantitative output. The cut-off FPR for discriminating between R5 and X4 use was set at 10%, adopting a modification of the European tropism guidelines, following observations that this threshold would not significantly affect R5 detection accuracy even by single testing.[12,15] X4 and dual/mixed (D/M) tropic viruses by Trofile or ESTA were cumulatively categorized as non-R5.

The baseline HIV-1 pol reverse transcriptase and protease genotype was processed by calculating the genotypic susceptibility score (GSS) using the latest available version from the Rega interpretation system (Rega 8.0.2) with respect to the antiretrovirals associated with maraviroc. We used the standard susceptible/intermediate/resistant categorization for all antiretrovirals used in combination with maraviroc, as by the output of Rega interpretation given by the HIVdb web-service (http://sierra2.stanford.edu/sierra/servlet/JSierra?action=hivalgs).

Individual drugs were assigned the following numerical susceptibility values, as suggested by the weighted Rega interpretation system: 0 for all drugs to which the virus was interpreted as resistant, 0.25 for intermediate resistant nevirapine and efavirenz, 0.5 for intermediate resistant nucleoside reverse transcriptase inhibitors (NRTIs), etravirine, and unboosted protease inhibitors (PIs), 0.75 for intermediate resistant boosted PIs, 1.0 for susceptible NRTIs, nonnucleoside reverse transcriptase inhibitors (NNRTIs), and unboosted PIs, and 1.5 for susceptible boosted PIs. Susceptibility to raltegravir and enfuvirtide was scored as 1 in case of first use of the drug or prior use in a virologically suppressive regimen and 0 in case of previous use but failure to suppress viral replication, conservatively assuming that the drugs had lost their antiviral activity. The arithmetic sum of the susceptibility scores of each drug associated with maraviroc was used to calculate the overall GSS of the cART regimen associated with maraviroc. Viral subtype was determined with automated BLAST analysis followed by manual phylogenetic analysis when the threshold of similarity to the best matching pure clade was below 95% or when the best matching clade was a circulating recombinant form (CRF). Unassigned subtypes were defined as undetermined.

Statistical analysis

Virological and immunological responses were assessed by survival analysis, as time to achieve an HIV-1 RNA of <50 copies/ml and time to achieve an increase of CD4$^+$ ≥100 cells/μl from baseline. Factors analyzed by univariable and multivariable Cox regression models as possibly associated with virological and immunological response included baseline HIV-1 RNA ($\log_{10}$ transformed, as a continuous variable),

the presence of non-R5 virus in at least one assay, type of tropism assay employed, nadir $CD4^+$ T cell count, baseline $CD4^+$ T cell count, GSS of concomitant regimens, and concomitant exposure to new antiretroviral drugs. Logistic regression was employed to analyze predictors of ever having a non-R5 tropism. Statistical analysis was performed by using SPSS version 18.0 (SPSS Inc., Chicago, IL).

Results

Baseline patients characteristics

A total of 191 ART regimens from 162 patients were eligible and analyzed. Table 1 summarizes patients' characteristics. One hundred and sixty-six patients (86.9%) carried viral subtype B, 16 (8.4%) non-B, and nine (4.7%) undetermined. The median (IQR) CD4 T cell count at baseline was 272/μl (130–442) and at nadir 78/μl (24–184) and the median HIV RNA at baseline was 4 $\log_{10}$ copies/ml (3.3–4.8).

The cases with baseline HIV-1 RNA ≥100,000 copies/ml were 36/191 (18.9%). The viral tropism was predicted by Trofile in 23.6% (45 treatments), by ESTA in 48.7% (93 treatments), by genotyping in 13.1% (25 treatments), and by a combination of genotyping and phenotyping in 14.6% (28 treatments). The median number of previously employed antiretroviral drugs was 11 (IQR 8–14) and the median number of previous antiretroviral lines was 10 (IQR 6–13).

Table 1. Characteristics of the Study Population ($n=191$)

Age (years)*	46 (IQR 42–51)
Gender, *n* (%)	Male 146 (76.4)
	Female 45 (23.6)
Nationality, *n* (%)	Italian 150 (78.5)
	Non-Italian 4 (2.1)
	Unknown 37 (19.4)
Risk group, *n* (%)	Heterosexual 62 (32.5)
	IDU 49 (25.7)
	Homosexual/bisexual 41 (21.5)
	Other/unknown 39 (20.3)
Viral subtype, *n* (%)	B 166 (86.9)
	Non-B 16 (8.4)
	Undetermined 9 (4.7)
Time (years) since HIV diagnosis*	16.4 (12.7–19.7)
Time (years) since starting cART*	12.2 (7.6–15.2)
CD4 baseline cells count (cells/μl)*	272 (130–442)
CD4 cells count nadir (cells/μl)*	78 (24–184)
HIV RNA at baseline ($\log_{10}$ copies/ml)*	4 (3.3–4.8)
Past antiretroviral drugs*	11 (IQR 8–14)
Past treatment lines*	10 (6–13)
GSS of the accompanying drugs, *n* (%)	
<1	29 (15.2)
1–<2	69 (36.1)
≥2	84 (44)
Unknown	9 (4.7)
Median*	1.75 (1–2.5)

Values are expressed as *n* (%) except for *median (IQR).
IDU, intravenous drug use; cART, combined antiretroviral treatment; GSS, genotypic susceptibility score.

Type of antiretrovirals used concomitantly with maraviroc, their calculated activity, and results of premaraviroc viral tropism

The maraviroc-based ART regimens included NRTIs in 55.5% (106/191) of cases, NNRTIs in 26.2% (50/191, including etravirine in 44/191, 23%), PIs in 69.6% (133/191) (with ritonavir boosting in 121/191, 63.4%, including darunavir in 94/191, 49.2%), raltegravir in 59.2% (113/191), and enfuvirtide in 11% (21/191). The median (IQR) GSS of the accompanying regimen was 1.75 (1–2.5): 15.2% (29 patients) presented GSS <1, 36.1% (69) GSS 1 to <2, and 44% (84) GSS ≥2; GSS was unknown in 4.7%.[9] The overall prevalences of R5, non-R5, and discordant strains were 92.2% (176/191), 4.7% (9/191), and 3.1% (6/191), respectively.

An R5 viral tropism was predicted in 176/191 (92.14%) of all cases analyzed; of these, 144/176 (81.8%) had baseline HIV-1 RNA <100,000 copies/ml and 32/176 (18.2%) had baseline HIV-1 RNA ≥100,000 copies/ml. In opposition, 144/155 (92.9%) with HIV-1 RNA <100,000 copies/ml and 32/36 (88.8%) with HIV-1 RNA ≥100,000 copies/ml were R5.

Overall, the R5 viral tropism was predicted for 45 of 47 isolates (95.7%) using Trofile, for 109 of 120 isolates (90.8%) using ESTA, and for 39 of 52 isolates (75%) using genotyping with G2P interpretation. Among the six discordant strains, five were classified as R5 tropic by ESTA and X4 tropic by G2P and one was classified as D/M tropic by ESTA and R5 by G2P.

The prevalence of non-R5 strains was 13.9% (11/79) among cases with baseline $CD4^+$ cell count <50 cell/μl, 7.1% (2/28) in cases with $CD4^+$ cell counts 50–100/μl, 4.8% (2/42) in cases with $CD4^+$ cell count 101–200/μl, and 0% (0/42) among those with $CD4^+$ cell counts >200/μl. The use of maraviroc in patients with non-R5 virus is partly justified by the deep salvage situation, whereby treating clinicians expected a residual antiviral activity and immunological activity of the drug, while lacking sufficiently active residual drug options. A lower nadir $CD4^+$ cell count ($p=0.01$) and a lower baseline $CD4^+$ cell count ($p=0.016$) were the only factors independently associated with non-R5 viral tropism by any assay.

Virological response and its predictors

During a median follow-up of 8 weeks (IQR 4–19), virological success was achieved in 65.4% of cases (in 68.4% among those with baseline HIV-1 RNA <100,000 copies/ml and in 58.3% among those with baseline HIV-1 RNA ≥100,000 copies/ml). The estimated proportion achieving virological suppression at 24 weeks of treatment was 74% for patients with baseline HIV-1 RNA <100,000 copies/ml and 66% for patients with baseline HIV-1 RNA ≥100,000 copies/ml (see Fig. 1a). The estimated virological response was similar in patients whose virus was classified as R5 and in patients with non-R5 tropic strains by any assay (see Fig. 1b). Moreover, estimated proportions with 12-week response were higher, although not significantly, in patients with discordant tropism results using different assays as compared to patients with concordant non-R5 tropic strains (37% vs. 17%, see Fig. 1c). Interestingly, the estimated virological response was remarkably similar, regardless of whether the viral tropism was screened using a genotypic assay, a phenotypic assay, or both assays (see Fig. 1d and e and Table 2).

Multivariable analysis showed a borderline association of higher accompanying drugs GSS (HR per 1 higher 1.21; 95%

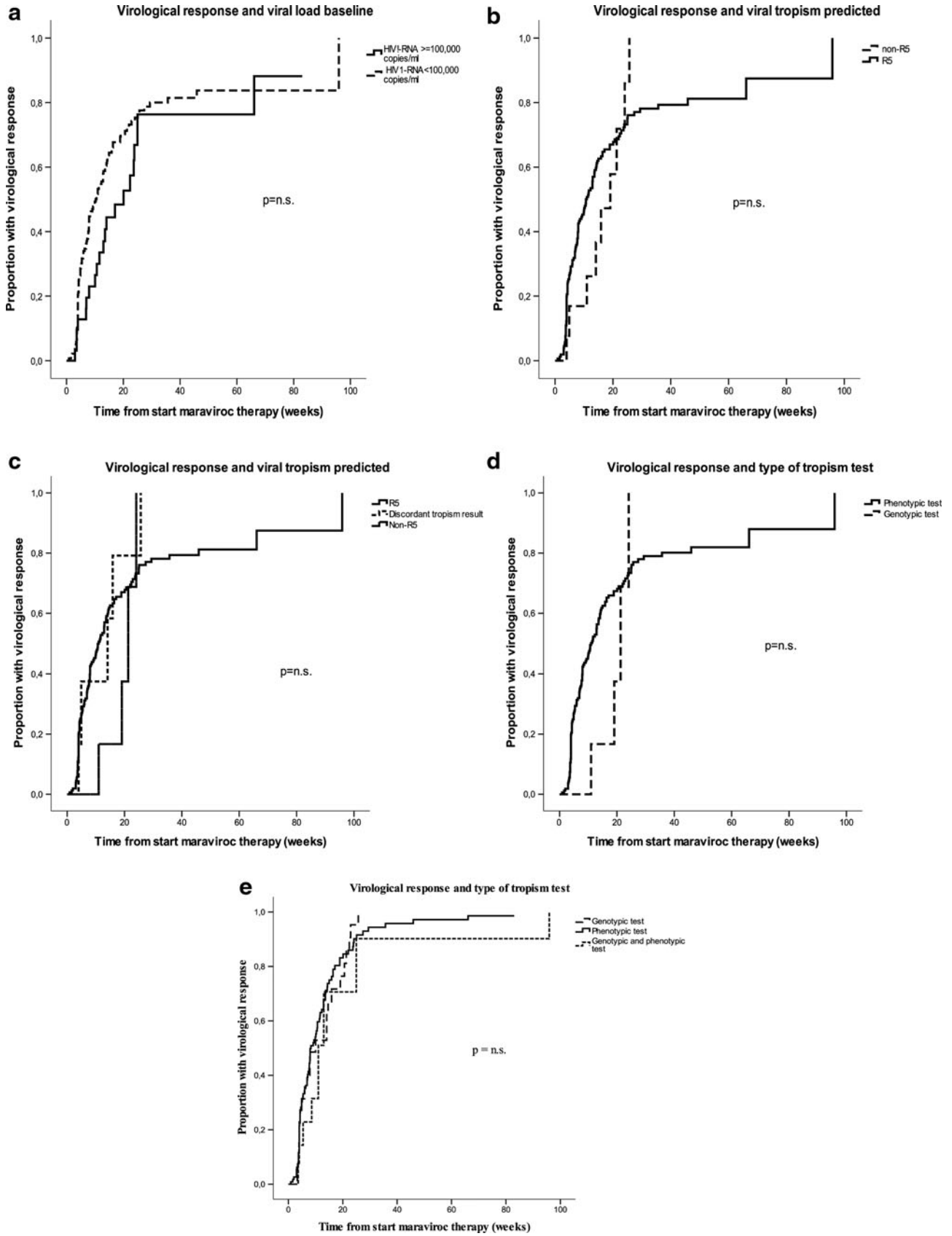

FIG. 1. Kaplan–Meier plots showing the time to achieve a virological response (HIV-1 RNA <50 copies/ml) by **(a)** baseline viral load, **(b,c)** tropism tests results, and **(d,e)** type of tropism testing used to screen patients. *p*-values by the log-rank test are shown.

Table 2. Univariable and Multivariable Cox Proportional Hazard Model Showing Relative Hazards for Virological Response, Fitted on the Whole Study Population ($n = 191$)

	Univariable analysis			*Multivariable analysis*		
Variable	*RH*	*95% CI*	p	*RH*	*95% CI*	p
Baseline HIV-1 RNA (per $\log_{10}$ copies/ml higher)	0.87	0.74–1.03	0.10	0.86	0.73–1.02	0.08
Non-R5 tropism versus R5	1.16	0.59–2.30	0.65	n.e.	n.e.	n.e.
GSS of concomitant drugs (per unit increase)	1.20	0.99–1.46	0.06	1.21	1.00–1.47	0.05
Concomitant boosted PI use	1.32	0.91–1.91	0.13	n.e.	n.e.	n.e.
Concomitant raltegravir use	1.23	0.86–1.78	0.25	n.e.	n.e.	n.e.
Type of tropism assay						
TROFILE (ref)	1.00		0.60	n.e.	n.e.	n.e.
ESTA	1.80	0.43–2.70	0.85	n.e.	n.e.	n.e.
Genotypic test	0.55	0.20–1.51	0.25	n.e.	n.e.	n.e.
Genotypic and phenotypic test	0.77	0.53–1.12	0.17	n.e.	n.e.	n.e.

RH, relative hazard; CI, confidence interval; ESTA, enhanced sensitivity trofile assay; GSS, genotypic susceptibility score; PI, protease inhibitor; n.e., not entered.

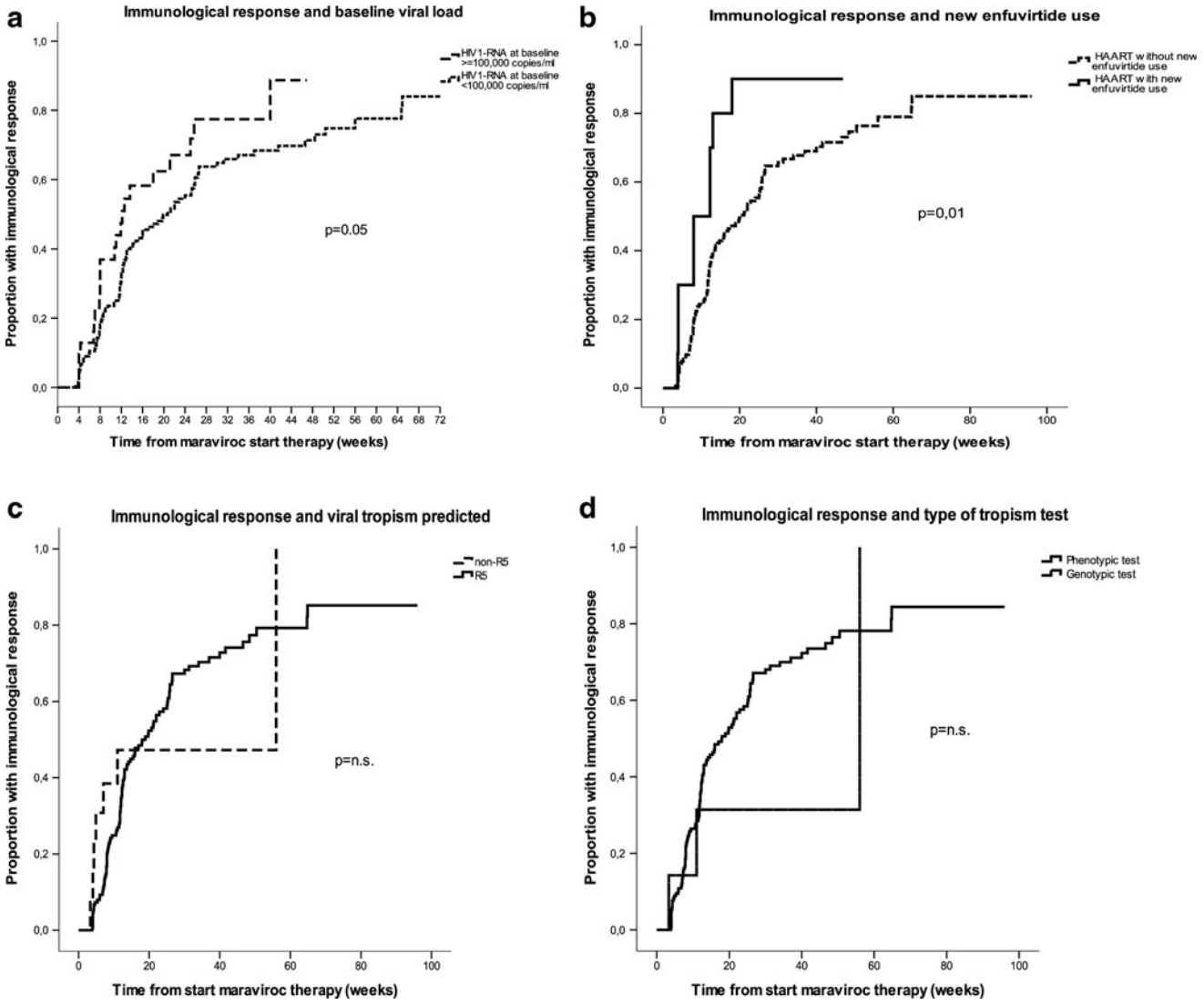

FIG. 2. Kaplan–Meier plots showing the time to achieve an immunological response (CD4 cell count increase of >100 cells/μl from baseline) by **(a)** baseline viral load stratum, **(b)** enfuvirtide use, **(c)** tropism tests results, and **(d)** type of tropism testing used to screen patients. *p*-values by the log-rank test are shown.

Table 3. Univariable and Multivariable Cox Proportional Hazard Model Showing Relative Hazards for Immunological Response, Fitted on the Whole Study Population ($n = 191$)

	Univariable analysis			*Multivariable analysis*		
Variable	*RH*	*95% CI*	p	*RH*	*95% CI*	p
Baseline HIV-1 RNA (per $\log_{10}$ copies/ml higher)	1.26	1.05–1.52	0.01	1.29	1.05–1.60	0.01
Enfuvirtide first use	2.30	1.16–4.58	0.01	2.05	0.96–4.39	0.06
Baseline CD4 (per 100 cells/μl higher)	1.05	0.97–1.14	0.19	n.e.	n.e.	n.e.
Non-R5 tropism vs. R5	1.08	0.50–2.33	0.77	n.e.	n.e.	n.e.
Type of tropism assay						
Genotypic test (ref)	1.00		0.55	n.e.	n.e.	n.e.
Phenotypic test	1.21	0.44–3.32	0.70	n.e.	n.e.	n.e.
Genotypic and phenotypic test	0.70	0.22–2.12	0.54	n.e.	n.e.	n.e.

RH, relative hazard; CI, confidence interval; n.e., not entered.

CI 1.00–1.47) and lower baseline viral load (HR per 1 log copies/ml higher 0.86; 0.73–1.02) with virological response.

Immunological response and its predictors

During a median follow-up of 12 weeks (IQR 7–25), immunological success was achieved in 58.1% of cases: 61.1% of those with baseline HIV-1 RNA $\geq$100,000 copies/ml and 57.4% of those with baseline HIV-1 RNA $<$100,000 copies/ml. After 24 weeks of treatment the estimated proportion of immunological responders was 56% among patients with baseline HIV-1 RNA $<$100,000 copies/ml and 67% among those with HIV-1 RNA $\geq$100,000 copies/ml (log-rank $p = 0.05$, see Fig. 2a). We also noted a significantly shorter time to immunological response in patients first using enfuvirtide as concomitant treatment (Fig. 2b). The new treatment with enfuvirtide was used in 10/191 patients (5.2%): all these cases had R5 viral tropism. At week 24 the immunological response estimate was 90% among patients treated with enfuvirtide and 66% among those without enfuvirtide (log-rank $p = 0.01$). Viral tropism assay types and results were not associated with time to immunological response (see Fig. 2c and d and Table 3). Multivariable Cox analysis revealed that higher baseline HIV-1 RNA ($p = 0.015$ HR per log copies/ml higher 1.29, 95% CI 1.05–1.60) and concomitant therapy with enfuvirtide ($p = 0.06$, HR 2.05, 0.96–4.39) were independently associated with shorter time to immunological response (see Table 3).

Evolution of viral tropism

After more than 1 month of maraviroc treatment at least one follow-up tropism test result was available for 20 treatments. These patients represent a subset of the patients with virological failure on maraviroc: no particular reason for genotyping this subset was found, except for the attitude of the site or clinician, since these patients were not different in terms of viral load, CD4, or GSS from the others who were failing (not shown).

Table 4. Comparison of Viral Tropism Before and After Maraviroc Treatment ($n = 20$)

Baseline and follow-up assay used	*Tropism evolution*	*Number of cases*
ESTA → G2P	R5 → R5	4
ESTA → ESTA	R5 → R5	1
ESTA, G2P → G2P	R5 → R5	4
G2P → ESTA	R5 → R5	1
ESTA → G2P	R5 → non-R5	6
ESTA → G2P, OTA	R5 → non-R5	1
G2P, ESTA → ESTA	DISC → non-R5	3

ESTA, enhanced sensitivity Trofile assay; G2P, geno2pheno.

The type of assays employed and the longitudinal tropism evolution results are summarized in Table 4. The baseline tropism was R5 in 17 (85%) cases, four of them by two different assays, and discordant in three cases (15%), all by different assays. The follow-up tropism was determined by a genotypic assay in 70% (14/20), by a phenotypic assay in 25% (5/20), and by a genotypic and phenotypic assay in 5% (1/20). The median time between the baseline and follow-up assays was 312 days (IQR 141.2–525.2) and the median level of viral load at the follow-up test was 3.8 $\log_{10}$ copies/ml (2.66–4.25). Changes in predicted coreceptor use were observed in 7 of 17 (41%) cases with R5 virus at baseline, which showed a tropism switch to non-R5 (see Table 4 for details).

Discussion

In the present study we investigated the impact of the use of maraviroc-containing regimens in clinical practice in a population of HIV-1-infected patients with a long infection history and past use of several antiretroviral drug classes. Even if all but three patients included were screened for the presence of R5-tropic virus, non-R5 tropism was still found in a small proportion of the sample. This finding can be explained by the fact that in selected cases clinicians might have attempted to exploit a residual virological or immunological activity of maraviroc in the context of a salvage ART regimen. In line with other studies,[3,16–21] the non-R5 viral tropism was correlated with a lower CD4 count at baseline and especially at nadir.

We found that a significant proportion of experienced patients treated with regimens containing maraviroc achieved a virological response. In agreement with previous observations,[3] the virological response rate showed a tendency to be higher in the group with lower baseline viral load than in the group with higher baseline viral load,[3] and in cases with a higher activity of the accompanying drugs, as estimated by the interpreted genotypic resistance assay results. Notably, the type of tropism assay used was not associated with virological response nor was it associated with immunological response. This confirms previous clinical trial-based

observations suggesting that both genotypic and phenotypic R5 tropism are associated with virological response.[22–25] In addition, in previous studies genotypic tropism tests were retrospectively validated, while here we show that selection of prospective maraviroc regimens using genotypic or phenotypic assays is associated with similar virological and immunological responses. In contrast with expectations, non-R5 tropism was not negatively associated with virological response. This might be due to the limited sample size of the category with non-R5 virus and might have been confounded by the relatively good activity of the accompanying drugs.

We found that more than half of experienced patients treated with regimens containing maraviroc achieved an immunological response. The immunological response rate was significantly higher in the group with higher baseline viral load. Interestingly, we observed a higher immunological response during concomitant treatment with enfuvirtide, both overall and when this drug was used for the first time (Table 3). To our knowledge, this is the first demonstration of an additive immunological effect of this drug in the context of maraviroc treatment. This observation is in line with an earlier *in vitro* study showing a positive correlation between low CCR5 density levels on CD4 T cells and increased sensitivity of R5 HIV-1 strains to enfuvirtide.[22] Enfuvirtide and maraviroc have shown a potential for synergistic antiviral activity *in vitro* and *in vivo*. Nonetheless, we observed an immunological benefit but not a virological advantage when using this combination *in vivo*. In agreement with our finding, previous *in vivo* studies have demonstrated a significant immunological benefit with enfuvirtide, which was at least partly independent of its antiviral activity.[26–29]

Among patients with pure R5 strains before maraviroc therapy, half showed a tropism shift from R5 to non-R5 at failure. An expansion of preexisting X4 or dual-tropic (R5X4) variants during clinical use of small molecule CCR5 inhibitors has been described in clinical trials.[30] This is in line with previous observations made using phenotypic assays and suggests that the potential use of the cheaper and more practical genotypic assays for interpreting the cause of virological failure with CCR5 antagonists and making informed decisions regarding their interruption deserves further evaluation. These results, however, must be interpreted with caution given the fact that tropism was determined only in part of the maraviroc failures, which cannot exclude selection bias, and that different assays for tropism determination were employed at baseline and during follow-up, although concordance between genotyping, and Trofile or ESTA is expected to be high (80% or higher).[25,31]

In conclusion, we observed significant virological and immunological responses with maraviroc-based regimens in antiretroviral-experienced patients. Any of the approved methods for determining R5 tropism is suitable for predicting virological and immunological responses in a real world setting. The potential for an additional immunological benefit of enfuvirtide in this context might be of clinical relevance and warrants a prospective evaluation.

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