First prospective comparison of genotypic versus phenotypic tropism assays in predicting virologic responses to maraviroc in a phase 3 study

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SUMMARY

Maraviroc (MVC, a CCR5 antagonist) is only fully active against CCR5 tropic [R5] HIV-1, and tropism testing is required prior to initiating treatment. The MODERN study prospectively compared genotypic (GTT) and phenotypic (Trofile®) tropism testing with treatment-naive HIV-1–infected participants randomized 1:1 to either GTT or Trofile tropism assessments. Participants with R5 virus were randomized 1:1 to receive darunavir/ritonavir (DRV/r) with either MVC or tenofovir/emtricitabine. Screening samples were also retrospectively tested using the alternative assay. Positive predictive values (PPVs) for each assay were estimated using both the observed MVC+DRV/r response rate (HIV-1 RNA <50 copies/mL at Week 48) and model-based response estimates. The observed MVC+DRV/r response rate was 146/181 (80.7%) for GTT versus 160/215 (74.4%) for Trofile, with a stratification adjusted difference of 6.6% (95% CI, –1.5% to 14.7%) in favor of GTT. The model-based PPV estimates (±standard error) were 80.5% (±2.38) and 78.0% (±2.35) for GTT and Trofile, respectively (difference, 2.5%; 95% CI, –2.0% to 7.0%). Most participants had R5 results using both assays (285/396; 72%) and, of those, 79.3% (226/285) had HIV-1 RNA <50 copies/mL at Week 48. Both the genotypic and phenotypic tropism assays evaluated can effectively predict treatment response to MVC.

INTRODUCTION

The first step in the process of human immunodeficiency virus type 1 (HIV-1) entry into the cell is binding of virus envelope glycoprotein (gp) 120 to CD4 on the cell surface. This leads to a conformational change in gp120 that allows binding to a chemokine coreceptor, either CC chemokine receptor 5 (CCR5) or CXC chemokine receptor 4 (CXCR4) (Moore and Doms, 2003). The virus population from an individual patient can therefore potentially contain viruses with different coreceptor tropism: only obligate CCR5-tropic (R5) or CXCR4-tropic (X4) viruses, dual-tropic viruses (R5X4) that can bind both CCR5 and CXCR4, or a heterogenous population of viruses with different tropism (dual/mixed [DM] tropism) (Westby *et al.*, 2006). Maraviroc (MVC) is a CCR5 antagonist and is fully active only against R5 viruses; partial activity against R5X4/DM viruses may be observed whereas X4 viruses are inherently not susceptible to MVC. Testing for tropism is therefore required prior to the use of MVC.

HIV-1 tropism can be assessed either through phenotypic assays (where pseudotyped virus is used to infect target cells expressing either CCR5 or CXCR4) or genotypic assays. The most commonly used phenotypic assay is the Trofile® assay (enhanced sensitivity version) (Cashin *et al.*, 2015). Genotypic assessment is based on the HIV-1 gp120 variable loop 3 (V3) sequence together with a bio-informatics algorithm to predict tropism (van der Ryst *et al.*, 2015). The most widely used algorithm for coreceptor tropism prediction is the open access "Geno2Pheno coreceptor" algorithm (http://coreceptor.geno2pheno.org/).

At the time of initiation of the MVC clinical development program, the phenotypic Trofile assay (original version) was the only tropism assay available to select participants for inclusion in Phase 3 registrational studies. The data from the MOTIVATE studies in treatment-experienced HIV-1–infected participants with R5 virus demonstrated a significant treatment benefit for MVC plus an optimized background treatment regimen (OBT) compared to OBT alone (Gulick *et al.*, 2008). In contrast, in study A4001029 in participants with non-R5 virus, no treatment benefit for MVC was observed (Saag *et al.*, 2009). Together, the data from these studies demonstrated the utility of the Trofile phenotypic assay in selecting appropriate patients for treatment with MVC. An enhanced sensitivity version of the Trofile assay (Reeves *et al.*, 2009) became available during the conduct of the MERIT study of MVC versus efa-

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virenz (both in combination with zidovudine/lamivudine) in treatment-naive participants with R5 virus. Screening samples of participants identified as R5 by the original Trofile assay were retested for tropism using the enhanced sensitivity version. A post-hoc analysis excluding the participants found to have non-R5 virus by the enhanced sensitivity assay reported improved response rates in the MVC treatment group, demonstrating the importance of assay sensitivity (Cooper et al., 2010).

The complexity of phenotypic assays creates significant barriers to their informing appropriate use of MVC in routine patient care; for example, requiring use of sophisticated laboratory facilities with associated logistical challenges, assay turnaround times and cost (van der Ryst et al., 2015). Genotypic assays provide a cheaper and more practical alternative to evaluate tropism. HIV-1 V3 loop sequencing can be performed in local/regional laboratories, and open access bio-informatics predictive algorithms such as "Geno2Pheno coreceptor" are available free of charge. Retrospective analyses of samples from the MERIT, MOTIVATE, A4001029 and other studies with MVC have demonstrated a high degree of concordance between genotypic (using both population-based Sanger sequencing and ultra-deep sequencing) and phenotypic assays (Swenson et al., 2011; McGovern et al., 2012; Kagan et al., 2012; Portsmouth et al., 2013; van der Ryst et al., 2015). However, no large clinical studies evaluating genotypic versus phenotypic tropism determination in a prospective fashion have been published.

The MODERN (Maraviroc Once-daily with Darunavir Enhanced by Ritonavir in a New regimen) study was designed to assess the safety and efficacy of MVC 150 mg once daily versus tenofovir/emtricitabine (TDF/FTC), both in combination with darunavir/ritonavir (DRV/r), in antiretroviral-naive participants infected with R5 virus (Stellbrink et al., 2016). The primary efficacy and safety results were previously reported; the trial was discontinued because of inferiority of the MVC treatment arm at 48 weeks. MODERN was also designed to prospectively evaluate a genotypic method to determine tropism versus a phenotypic method–the results of this secondary analysis are discussed here.

MATERIALS AND METHODS

Study design and population

MODERN (A4001095) was a multicenter, Phase 3 study conducted in the European Union, United States of America, Australia, and Canada. The study design is described in detail elsewhere (Stellbrink et al., 2016). Briefly, HIV-1–infected, antiretroviral-naive adults with plasma HIV-1 RNA ≥1000 copies/mL and no evidence of reduced susceptibility to DRV, TDF, or FTC were screened for the study. A blinded 2-stage randomization scheme was used, with participants first randomized 1:1 to undergo either genotypic (Siemens genotypic tropism test [GTT]) or phenotypic (enhanced sensitivity Trofile assay) tropism testing. Tropism of screening samples from enrolled participants was also retrospectively determined using the alternate assay to that used at screening, so that all samples were tested using both assays. Participants identified as having R5 HIV-1 by the designated assay were then randomized 1:1 to receive MVC 150 mg once daily or TDF/FTC once daily each with DRV/r once daily for 96 weeks, with stratification by screening plasma HIV-1 RNA (≥ or <100,000 copies/mL) (Figure 1). Both the tropism assay and the treatment assignment were blinded. After Screening and Baseline, visits were scheduled at Weeks 4, 8, 12, 16, 20, 24, 36, 48, 60, 72, 84, 96, and follow-up (28 days after last study dose). Virologic and immunologic efficacy and safety were assessed as described by Stellbrink et al. (Stellbrink et al., 2016).

The primary endpoint was the proportion of participants with HIV-1 RNA <50 copies/mL (Food and Drug Administration snapshot algorithm where missing,
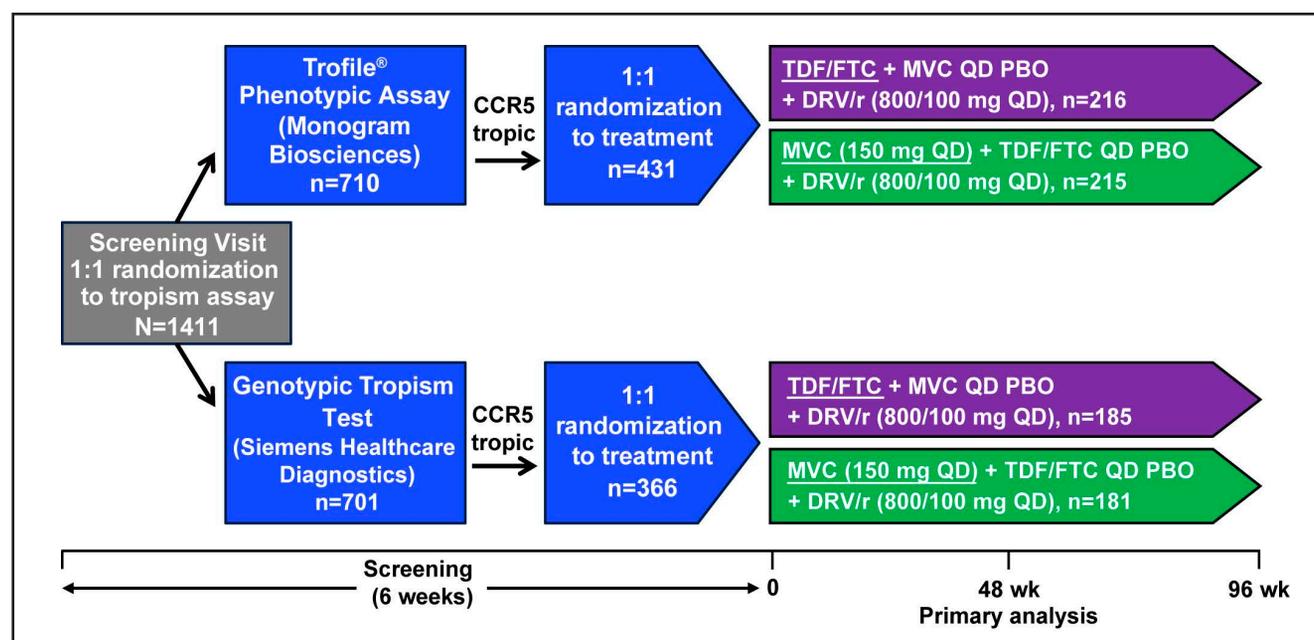

Figure 1 - *MODERN study design schematic. Of the 813 participants randomized, only 797 were treated (Stellbrink et al., 2016). DRV/r, darunavir/ritonavir; MVC, maraviroc; PBO, placebo; QD, once daily; TDF/FTC, tenofovir/emtricitabine.*

switched, or discontinued equals failure [MSDF]) at Week 48. The relationship between both the genotypic and phenotypic assays and virologic success (as defined by HIV-1 RNA <50 copies/mL) at Week 48 (described here) was a secondary endpoint.

Virologic failure was defined as meeting one of the following criteria (confirmed in a subsequent sample): decrease in HIV-1 RNA <1 log_{10} from baseline after Week 4 unless HIV-1 RNA was <50 copies/mL; HIV-1 RNA >1 log_{10} above the nadir value after Week 4; HIV-1 RNA ≥50 copies/mL at any time after Week 24; HIV-1 RNA ≥50 copies/mL after suppression to <50 copies/mL on 2 consecutive visits; or decrease in HIV-1 RNA 2 log_{10} or less from baseline on or after Week 12 unless HIV-1 RNA was <400 copies/mL (Stellbrink *et al.*, 2016). Samples from participants with protocol-defined treatment failure (PDTF) were assessed for resistance to study drugs and tropism. The relationship between PDTF and tropism screening assay was also assessed.

The study was registered with ClinicalTrials.gov (NCT01345630). It was conducted in compliance with the Declaration of Helsinki and International Conference on Harmonisation Good Clinical Practice Guidelines and all local regulatory requirements were followed. The study protocol was approved by institutional review boards or independent ethics committees at all sites and written informed consent was provided by all participants.

Assays

Plasma HIV-1 RNA concentration was determined using the Real Time HIV-1 Viral Load assay with a lower limit of quantification of 40 copies/mL (Abbott, Des Plaines, IL, USA).

For phenotypic tropism determination the (enhanced sensitivity) Trofile assay from Monogram Biosciences (South San Francisco, CA, USA) was used.

Genotypic tropism determination was done using the Siemens Healthcare Diagnostics GTT (Berkeley, CA, USA) laboratory developed test (LDT). For this assay, the HIV-1 gp120 V3 loop sequence was determined using population-based Sanger sequencing and tropism was predicted using the Geno2Pheno algorithm (https://coreceptor.geno2pheno.org/) to assess triplicate sequences with the false positive rate (FPR) set to 10%.

Statistical analysis

The relative performance of the genotypic and phenotypic tropism assays was compared in several ways.

First, for each tropism assay in turn, the percentage of participants with HIV-1 RNA <50 copies/mL (MSDF) at Week 48 in the full analysis set was estimated for R5 participants as determined by the assay. The difference in the percentages between the MVC and the TDF/FTC treatment arms and the 2-sided 95% confidence interval for the difference was provided using the stratum-adjusted Mantel-Haenszel method (Koch *et al.*, 1998). The estimate was adjusted for the screening plasma HIV-1 RNA level (<100,000 vs ≥100,000 copies/mL).

Second, to assess the utility of GTT compared to Trofile, the positive predictive value (PPV) for the 2 assays was compared amongst MVC-treated participants. The PPV of an assay was defined as the expected proportion of participants in the MVC treatment group with HIV-1 RNA <50 copies/mL (MSDF) at Week 48 among participants who were identified as having R5 virus at screening by that as-say. Maraviroc treatment outcomes in participants determined to have R5 virus by GTT was compared to TDF/FTC results among participants determined to have R5 virus by either assay.

Concordance between the 2 assays was evaluated by analyzing the percentage agreement of tropism calls (R5 or non-R5) between the assays using the Maximum Likelihood (ML) method and including all screening data, as well as data from the retesting of screening samples from all participants randomized to the MVC treatment group; non-R5 included DM and/or X4 tropism.

To improve precision of the estimated treatment effect and PPV difference, the screening samples from all enrolled participants were retested using the alternative assay. The PPV estimates for each assay were then based on 2 sets of participants, those originally randomized to the assay and those randomized to the alternative assay, but on retesting were also identified with R5 virus using the original assay. As the clinical outcome could potentially be different between the 2 subsets of participants for each assay, ML estimator was a weighted average of the 2 types, thereby removing any effect of the alternative assay on the estimate for the original assay. The estimation made use of all tropism data to improve the precision of the weighting. To account for the stratification by screening plasma HIV-1 RNA ≥ or <100,000 copies/mL, the stratum-adjusted Mantel-Haenszel method was used (Koch *et al.*, 1989). For these model-based estimates, samples with a non-reportable result were treated as non-R5. Relative assay performance was then assessed by difference in PPV (with 95% CI) at Week 48 between the genotypic and phenotypic tropism assays in the MVC and TDF/FTC treatment groups using the ML method and including tropism retesting data of all participants. The difference in proportions (with 2-sided 95% CIs) of participants with HIV-1 RNA <50 copies/mL between the MVC and TDF/FTC treatment groups in the subset of participants randomized to the genotypic and phenotypic assays was also assessed. Subgroup analyses by screening HIV-1 RNA (<100,000 copies/mL vs ≥100,000 copies/mL), screening CD4 count (<200 cells/mm³, ≥200 to <350 cells/mm³, ≥350 to <500 cells/mm³ and ≥500 cells/mm³) and gender were performed.

RESULTS

Screening tropism results and treatment population

A total of 1423 participants were screened for this study. Of these, 710 were tested for tropism using Trofile, and 701 were tested for tropism using GTT. A total of 610 participants failed screening; of these, 264 (103 with Trofile and 161 with GTT) had non-reportable tropism results and 220 (99 with Trofile and 121 with GTT) had non-R5 virus, while 126 were excluded for other reasons, including meeting other exclusion criteria, no longer willing to participate, lost to follow-up, protocol deviation, or adverse event. Samples from 12 participants (6 per each assay) who failed screening could not be evaluated.

The remaining 813 participants with R5 virus were randomized to either of the 2 treatment arms (408 to MVC+DRV/r, and 405 to TDF/FTC+DRV/r), but only 797 received study drug; 396 in the MVC+DRV/r treatment group, and 401 in the TDF/FTC+DRV/r group (*Figure 1*). Of the 396 MVC-treated participants, 215 were identified

Table 1 - *Demographic and clinical characteristics by randomized assay and randomized treatment group.*

	Randomized to genotype assay			Randomized to Trofile assay		
	MVC+ DRV/r (n=187)	*TDF/FTC+ DRV/r (n=185)*	*Total* (N=702)*	*MVC+ DRV/r (n=221)*	*TDF/FTC+ DRV/r (n=220)*	*Total* (N=707)*
Screening HIV-1 RNA, n (%)						
<100,000 copies/mL	149 (79.7)	146 (78.9)	545 (77.6)	184 (83.3)	183 (83.2)	581 (82.2)
≥100,000 copies/mL	38 (20.3)	39 (21.1)	155 (22.1)	37 (16.7)	37 (16.8)	124 (17.5)
Screening CD4 counts, n (%)						
<200 cells/mm^3	15 (8.0)	20 (10.8)	110 (15.7)	19 (8.6)	28 (12.7)	94 (13.3)
200-350 cells/mm^3	82 (43.9)	70 (37.8)	277 (39.5)	84 (38.0)	79 (35.9)	254 (35.9)
>350-500 cells/mm^3	52 (27.8)	49 (26.5)	185 (26.4)	79 (35.8)	69 (31.4)	227 (32.1)
>500 cells/mm^3	33 (17.7)	46 (24.9)	125 (17.8)	36 (16.3)	42 (19.1)	125 (17.7)
Gender, n (%)						
Female	18 (9.6)	16 (8.7)	64 (9.1)	20 (9.1)	18 (8.2)	69 (9.8)
Male	169 (90.4)	169 (91.4)	638 (90.9)	201 (90.9)	202 (91.8)	638 (90.2)
Race, n (%)						
White	158 (84.5)	151 (81.6)	565 (80.5)	174 (78.7)	178 (80.9)	558 (78.9)
Black	25 (13.4)	24 (13.0)	111 (15.8)	37 (16.7)	32 (14.6)	116 (16.4)
Asian	2 (1.1)	6 (3.2)	14 (2.0)	3 (1.4)	3 (1.4)	13 (1.8)
Other	2 (1.1)	4 (2.2)	12 (1.7)	7 (3.2)	7 (3.2)	20 (2.8)
Age, mean ± SD, y	37.0±10.3	35.8±10.8	36.8±10.6	38.7±11.2	36.5±11.0	37.2±10.7

*The sum of MVC+DRV/r and TDF/FTC+DRV/r columns do not add up to the total within each randomized assay as only subjects with R5 virus were randomized to one of the 2 treatment arms. DRV/r, darunavir/ritonavir; MVC, maraviroc; SD, standard deviation; TDF/FTC, tenofovir/emtricitabine.

as having R5 virus by Trofile, and 181 were identified as having R5 virus by GTT. In the TDF/FTC treatment group 216 were identified as having R5 virus by Trofile, and 185 were identified as having R5 virus by GTT. Baseline demographic data were similar between the 2 treatment groups (*Table 1*) (Stellbrink *et al.*, 2016). Similarly, baseline data between the groups assigned to each tropism assay were similar (*Table 1*).

Primary efficacy and safety results

The primary efficacy and safety results are described elsewhere in detail (Stellbrink *et al.*, 2016). Briefly, the proportion of participants who met the primary endpoint of HIV-1 RNA <50 copies/mL at Week 48 was 77.3% for MVC+DRV/r and 86.8% for TDF/FTC+DRV/r, with more MVC-treated participants experiencing PDTF. The stratification adjusted treatment difference was –9.5% (95% CI, –14.8% to –4.2%). MVC+DRV/r did not meet the pre-specified non-inferiority criteria (10%). The study was terminated early based on the inferiority of the MVC treatment arm and did not continue to the planned 96-week endpoint. MVC was well-tolerated and no new safety concerns were identified.

Comparison of Trofile and Siemens GTT assays
Observed response rate by randomized tropism assay

The observed MVC+DRV/r response rate was 146/181 (80.7%) for GTT versus 160/215 (74.4%) for Trofile, with a stratification adjusted difference between the assays of 6.6% in favor of GTT (95% CI, –1.5% to 14.7%). *Figure 2* illustrates the longitudinal virologic response rate for those participants prospectively randomized to MVC+DRV/r by either Trofile or GTT assay.

There was no difference in response rate between assays in the TDF/FTC+DRV/r arm; the observed TDF/FTC+DRV/r response rate was 160/185 (86.5%) for

Figure 2 - *Response rates (HIV-1 RNA <50 copies/mL) for participants randomized to receive MVC+TDF/FTC by randomization tropism assay. MVC, maraviroc; TDF/FTC, tenofovir/emtricitabine.*
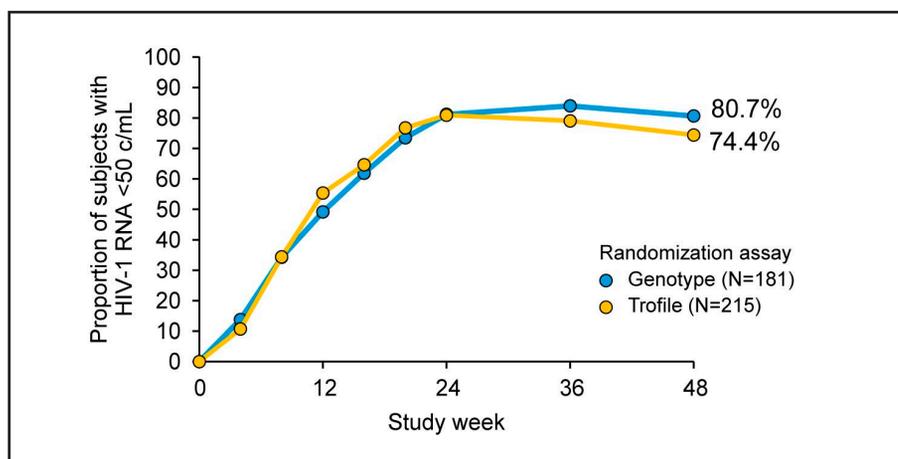
Table 2 - *MVC+DRV/r treatment outcomes at Week 48 by tropism results (including retrospective tropism data).*

MVC+DRV/r Week 48 response (HIV-1 RNA <50 copies/mL), n/N (%)		*Trofile*			*Total*
		R5	*Non-R5*	*Non-reportable*	
GTT	R5	226/285 (79.3)	11/14 (78.6)	14/19 (73.7)	251/318 (78.9)
	Non-R5	21/26 (80.8)	0	0	21/26 (80.8)
	Non-Reportable	34/52 (65.4)	0	0	34/52 (65.4)
Total		281/363 (77.4)	11/14 (78.6)	14/19 (73.7)	306/396 (77.3)

DRV/r, darunavir/ritonavir; GTT, Siemens genotypic tropism test; MVC, maraviroc.
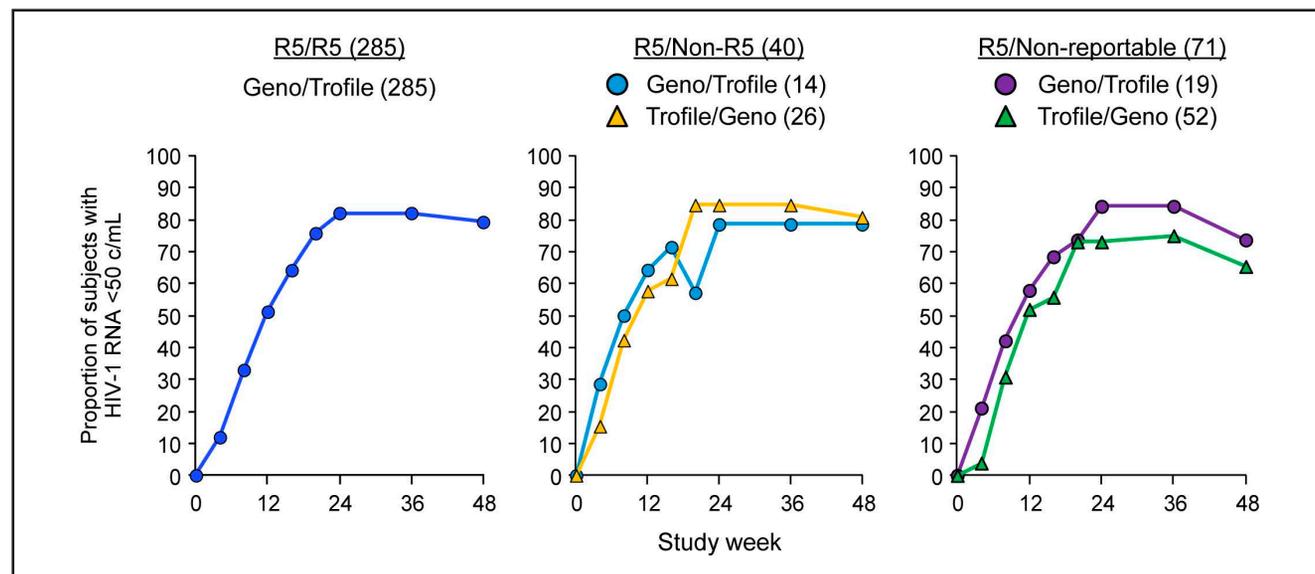

Figure 3 - *Longitudinal MVC+DRV/r response rates by tropism assay concordance. DRV/r, darunavir/ritonavir; MVC, maraviroc.*

GTT versus 188/216 (87.0%) for Trofile, with a stratification adjusted difference between the assays of –0.1% (95% CI, –6.8% to 6.6%).

Although the outcome in the MVC+DRV/r treatment group numerically favored GTT, there was no statistically significant difference in the proportion of participants meeting the primary endpoint (HIV-1 RNA <50 copies/mL) between the assays for either treatment group.

Retrospective evaluation of screening tropism and assay concordance

Most enrolled participants had R5 results at screening using both assays. In the TDF/FTC treatment group 290/401 (72%) had an R5 tropism result as determined by both assays. In the MVC treatment group 285/396 (72%) had concordant R5 results. For the 285 MVC-treated participants who had an R5 tropism result by both assays, approximately 80% achieved HIV-1 RNA <50 copies/mL at Week 48. Response curves for the 40 participants that

were R5 by one assay but non-R5 by the other overlap, with approximately 80% of participants achieving HIV-1 RNA <50 copies/mL at Week 48. The lowest response rates were seen for participants defined as R5 by one assay and non-reportable by the other, with 74% and 65% of participants with HIV-1 RNA <50 copies/mL at Week 48, for GTT/Trofile and Trofile/GTT, respectively (*Table 2* and *Figure 3*). The small numbers in the non-concordant groups preclude any conclusions regarding the significance of the differences observed.

Model-based PPV estimates

The model-based estimates of PPV (±standard error [SE]) in the MVC treatment group were 80.5% (±2.38) and 78.0% (±2.35) for GTT and Trofile, respectively (difference=2.48%; 95% CI, –2.02% to 6.98%). In the TDF/FTC group, model-based estimates of PPV (±SE) were 88.0% (±1.78) for GTT and 88.2% (±1.80) for Trofile, with a point estimate for the difference of –0.20% (95% CI, –3.31% to

Table 3 - *Summary of screening randomization assays used in participants with evaluable protocol-defined treatment failure outcomes at Week 48.*

Treatment group	Confirmed PDTF	Evaluable PDTF*	Screening randomization assay	
			GTT	*Trofile*
MVC+DRV/r	37[†]	17	7	10
TDF/FTC+DRV/r	10[†]	3	2	1

*Participants with confirmed PDTF and plasma HIV-1 RNA >400 copies/mL while on treatment at or after failure. [†]Excludes 3 participants from each treatment group who continued on treatment after confirmed PDTF and later responded to therapy. DRV/r, darunavir/ritonavir; GTT, Siemens genotypic tropism test; MVC, maraviroc; PDTF, protocol-defined treatment failure; TDF/FTC, tenofovir/emtricitabine.

2.92%). There were also no statistically significant differences in point estimates for PPVs between the assays for the subgroup analyses by screening HIV-1 RNA, CD4 count or gender.

For GTT the difference in proportion of participants with HIV-1 RNA <50 copies/mL between the MVC and TDF/FTC treatment groups were –6.15 (95% CI, –13.66 to 1.36), while for Trofile the difference was –12.61 (95% CI, –20.03 to –5.20).

Virologic failure and screening tropism assay
Evaluable participants included those with confirmed PDTF and plasma HIV-1 RNA >400 copies/mL while on treatment at or after failure (driven by assay limitations for resistance and tropism testing) (ePDTF population). A total of 20 participants met ePDTF criteria across both treatment groups (Stellbrink *et al.*, 2016). There was no apparent effect of the screening tropism assay. For those who received MVC+DRV/r, 7 were randomized on the basis of GTT and 10 with Trofile. For TDF/FTC+DRV/r, 2 were randomized by GTT and 1 was randomized by Trofile (*Table 3*).

DISCUSSION

MODERN was the first large Phase 3 clinical trial in treatment-naive HIV-1 participants to prospectively compare the performance of a genotypic tropism test (Siemens Healthcare Diagnostics laboratory developed GTT) with a phenotypic tropism test (Monogram Biosciences enhanced sensitivity Trofile assay) to determine whether participants had R5 virus and would therefore be eligible for MVC treatment. Although there was a small numerical advantage for the genotypic assay, this did not reach statistical significance, and both the GTT and Trofile assays effectively predicted treatment response to MVC+DRV/r. It also confirms the results of a small prospective study in treatment-experienced participants demonstrating that the Trofile assay and genotypic tropism prediction (using population based V3-loop sequencing and interpretation with the Geno2Pheno algorithm) were equally effective in predicting a treatment response to MVC (Nozza *et al.*, 2016).

The retrospective testing of all samples in this study with the alternative assay allowed analysis of a larger dataset for each assay method, as well as assessment of concordance between the assays. Model-based PPV estimates incorporating data from both the prospective analysis by the original randomized assay, as well as the retrospective data from the alternate assay, also demonstrated little difference between the genotypic and phenotypic assay methods in predicting response to MVC. A high rate of concordance between the assays was observed, with 72% of samples being identified as having R5 virus by both assays. This is consistent with data from retrospective studies that generally showed a high rate of concordance between genotypic assays based on V3 loop sequencing and the enhanced sensitivity Trofile assay (Svicher *et al.*, 2010; Swenson *et al.*, 2011; McGovern *et al.*, 2012; Kagan *et al.*, 2012; Portsmouth *et al.*, 2013). A retrospective analysis of samples from the MERIT study of MVC in treatment-naive participants demonstrated 84% concordance between the Trofile assay and V3 loop sequence-based genotypic assessment (McGovern *et al.*, 2012). In the OSCAR study, a concordance of 88.2% (FPR of 10%) for detection of R5

virus between genotypic tropism testing and Trofile was demonstrated (Svicher *et al.*, 2010).

A previous study evaluating the PPV of V3 sequencing based genotypic tropism assessment versus Trofile in a retrospective fashion has demonstrated that population-based sequencing resulted in a lower PPV compared to Trofile, but that next-generation ultra-deep sequencing resulted in similar PPVs (Kagan, 2012). However, in the MODERN study, V3 loop population-based Sanger sequencing, using triplicate sequences and a Geno2Pheno FPR of 10%, was demonstrated to be adequately sensitive, resulting in a PPV similar to that of Trofile. The FPR of the Geno2Pheno algorithm is the probability of incorrectly classifying an R5 virus as X4–the higher the FPR, the more conservative the interpretation of the algorithm is (https://coreceptor.geno2pheno.org/). The 10% level selected here appears to represent an adequate balance between sensitivity and specificity for prediction of tropism from V3 loop sequences. Altogether, these examples highlight that genotypic concordance with phenotypic methods may vary depending upon different factors such as FPR used, single versus triplicate sequencing and sensitivity of sequencing methods for minor variants.

In conclusion, MODERN was the first large clinical study to prospectively evaluate a genotypic method to evaluate tropism prior to starting MVC therapy. Altogether the genotypic assay performed similar to the phenotypic assay and both assays appropriately identified participants for MVC therapy. These findings confirmed the results from a smaller prospective study (Nozza *et al.*, 2016) and other retrospective analyses (Svicher *et al.*, 2010; Swenson *et al.*, 2011; McGovern *et al.*, 2012; Kagan *et al.*, 2012; Portsmouth *et al.*, 2013). These reports demonstrate the suitability of using either genotypic or phenotypic assays to identify HIV-1–infected individuals who may benefit from MVC and are applicable to real-world clinical practice. In this study, MVC once daily plus DRV/r was inferior to TDF/FTC plus DRV/r and the study was terminated early because of lack of efficacy for the MVC+DRV/r arm. However, the analysis of tropism data presented here demonstrates that this could not be attributed to failure of either of the tropism assays to identify patients likely to benefit from MVC therapy and that other factors in addition to R5 tropism play a role in determining response to MVC.

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