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Correlation between genotypic (V3 population sequencing) and phenotypic (Trofile ES) methods of characterizing co-receptor usage of HIV-1 from 200 treatment-naïve HIV patients screened for Study A4001078

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ABSTRACT

Assessment of HIV-1 co-receptor usage is essential to identify patients who are likely to respond to maraviroc (MVC)-containing regimens. Co-receptor usage of plasma virus from all treatment-naïve patients screened for a MVC clinical trial was assessed using phenotypic and genotypic methodologies to evaluate concordance between testing methods and to assess the quantity of CXCR4-using (non-R5) virus in samples giving discordant results. Co-receptor usage was prospectively measured using the enhanced sensitivity Trofile assay (Trofile ES) to screen patients for enrollment in Study A4001078. Population and deep sequencing methodologies were utilized retrospectively to analyze all screening samples, with co-receptor usage determined using the geno2pheno algorithm. Concordance between methods was explored using descriptive statistics. The quantity of non-R5 virus in all samples was measured using deep sequencing. Trofile ES and matched genotype results were obtained for 199 screening samples. Concordance of Trofile ES with population genotyping (5.75% false-positive rate [FPR]) and deep sequencing (3.5% FPR; 2% non-R5 threshold) was 91.7% and 89.6%, respectively. Population genotype data were available for all samples with non-reportable Trofile ES results; the distribution of co-receptor usage in this set was consistent with that in the overall population: 75% (12/16) R5 and 25% (4/16) non-R5. The majority of samples contained non-R5 plasma HIV-1 RNA estimated at either <1 $\log_{10}$ (62.0%) or $\geqslant$4 $\log_{10}$ (30.5%) copies/mL; the absolute amount of detectable non-R5 virus remained stable between screening and baseline visits. Samples originally classified as non-R5 by Trofile ES but R5 by population sequencing had a relatively low absolute amount of non-R5 virus (mean 2.1%, median 0.1%). The determination of co-receptor usage using either Trofile ES or genotyping methodologies showed similar frequencies of R5 and non-R5 virus in this treatment-naïve study population. For both concordant and discordant samples, population sequencing appropriately identified R5 samples with low levels of non-R5-using virus.

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1. Introduction

The prevalence of CCR5-using (R5) HIV-1 is greater in treatment-naïve individuals (80–90%) compared with treatment-experienced individuals (50–55%), more of whom have CXCR4-using or dual/mixed virus (Hoffmann, 2007). As maraviroc (MVC), a CCR5 antagonist, inhibits CCR5-dependent HIV-1 cell entry (Dorr et al., 2005), determination of HIV-1 co-receptor usage is required before commencing treatment.

The original Trofile assay (Monogram Biosciences), based on recombinant virus technology, was the assay most widely used for prospective determination of co-receptor usage in clinical trials of the first CCR5 antagonists, including the registrational Phase 3 trials for MVC (Whitcomb et al., 2007). However, it has since been superseded by an enhanced sensitivity Trofile assay, denoted here

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; ES, enhanced sensitivity; FPR, false-positive rate; g2p, geno2pheno; MVC, maraviroc; NR, non-reportable; QD, once daily; sff, Standard Flowgram Format.

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as Trofile ES, which is more sensitive for detecting minor CXCR4-using (dual, and/or X4; non-R5) populations *in vitro* (Reeves et al., 2009). HIV-1 co-receptor usage is largely determined by the third variable region (V3) of the HIV envelope glycoprotein (Hwang et al., 1991). Genotypic methods, such as population genotyping or deep sequencing, are based on sequencing the V3 loop and analyzing the sequence using bioinformatic algorithms to infer likely co-receptor usage.

The determination of HIV-1 co-receptor usage using Trofile ES and genotypic methodologies has been previously shown to predict clinical response to MVC with similar degrees of accuracy (McGovern et al., 2010a,b; Swenson et al., 2011). Retrospective analyses of viral co-receptor usage in the MVC registrational studies MERIT (Portsmouth et al., 2010) and MOTIVATE (McGovern et al., 2010b) showed a relatively high degree of concordance, albeit in samples from patients enrolled in these studies. Co-receptor usage in screening samples from a randomized trial of MVC-based therapy (not preselected for R5 virus and prior to study enrollment and MVC administration) has not been assessed previously.

The objectives of this study were: to assess concordance between results obtained using Trofile ES and genotypic methodologies in screening samples from the MVC clinical trial Study A4001078 (Portsmouth et al., 2011); to quantify, using deep sequencing methods, the amount of non-R5 virus in all samples, particularly in those giving discordant results between genotypic and phenotypic assays; and to determine the co-receptor usage of virus from samples with a non-reportable (NR) result using Trofile ES, comparing the composition of this subset with distribution in the overall patient population.

2. Materials and methods

Study A4001078 was a randomized, open-label, two-arm, international Phase 2b study (Clinicaltrials.gov identifier NCT00827112) conducted at 33 centers in Germany, Spain, and the US. The study was carried out in accordance with the ethical principles set out in the Declaration of Helsinki, and the good clinical practice guidelines established by the International Conference on Harmonisation. All patients provided informed written consent for participation in Study A4001078 and retrospective determination of co-receptor usage.

Treatment-naïve patients infected with R5 HIV-1, as determined at the screening visit using Trofile ES, were randomized to receive atazanavir/ritonavir (ATV/r; 300/100 mg once daily [QD]) with either MVC (150 mg QD) or tenofovir/emtricitabine (Truvada; 300/200 mg QD) for 48 weeks.

Plasma HIV-1 RNA from each patient screened for study entry was re-assessed retrospectively for viral co-receptor usage at screening and baseline using both population and deep sequencing methodologies. Amplicons of 420 base-pairs, which included the encoding region for the entire V3 loop of gp120, were generated using the single nested RT-PCR product of viral RNA extracted from plasma. The population genotype of each sample was determined using standard Sanger sequencing (single sample) and co-receptor usage was assigned using the geno2pheno (co-receptor) algorithm (g2p) (Sing et al., 2007) with a false-positive rate (FPR, i.e. predicted frequency of classifying R5 virus as non-R5 virus) of 5.75% (or other FPRs as indicated).

The viral co-receptor usage composition of each sample was assessed using the GS FLX Titanium (454 Life Sciences/Roche) amplicon sequencing protocol. The HIV-1 V3 deep sequencing approach of the samples achieved an average ± standard deviation (range) of 11,490 ± 4646 (1148–27,714) reads per sample. Low numbers of viral input templates in the reverse transcription and subsequent PCR may have resulted in oversampling leading to pseudohomogeneity virtually not present in the virus population (Jabara et al., 2011; Vandenbroucke et al., 2010). The average viral load was 100,998 copies/mL (median: 46,500 copies/mL; range: 1650–750,000 copies/mL). Since HIV-1 RNA was extracted from 500 μl of plasma (QIAamp Viral RNA Mini-Kit, Qiagen, Hilden, Germany [protocol slightly modified]), the arithmetical range of input RNA molecules was 825–375,000.

Sequences were extracted directly from the Standard Flowgram Format (sff) files, which store the sequencing trace data produced by the 454 GS FLX System, analyzed and processed for full-length V3, and the co-receptor usage of each individual sequence inferred using g2p with an FPR of 3.5%. Samples were classified as non-R5 if at least 2% of individual sequences were inferred as non-R5. The non-R5 viremia (copies/mL) was estimated as the overall screening plasma HIV-1 RNA concentration (copies/mL) multiplied by the proportion of non-R5 sequences, as determined using deep sequencing. The quality of individual V3 loops was assessed using a 95-percentile cut-off as implemented in the g2p-454 algorithm (Sing et al., 2007).

Concordance between Trofile ES, population genotyping, and deep sequencing was explored using descriptive statistics; the quantity of non-R5 virus in screening and baseline samples both concordant and discordant, comparing genotype and phenotype, was evaluated using population and deep sequencing methodologies.

3. Results

Of the 220 patients who were screened for entry to Study A4001078, 200 patients had prospective Trofile ES screening data available (Supplementary Fig. 1). Screening samples from 20 patients were not included in this analysis due to patients not meeting entry criteria ($n = 4$), patients no longer willing to participate ($n = 6$), or other reasons ($n = 10$). Matched genotype data were obtained retrospectively for 199/200 patients (99.5%). Demographic data are not available for patients that screen-failed, although for the 199 patients with matching Trofile ES and genotypic data, the median plasma HIV-1 RNA concentration was 46,500 copies/ml (range 1670–750,000 copies/ml). One-hundred-and-twenty-one patients infected with R5 HIV-1, determined by Trofile ES, were randomized to study treatment (Supplementary Fig. 1).

Co-receptor usage results for the 199 patients with Trofile ES and genotype data are presented in Fig. 1A. A greater percentage of patient samples were characterized as R5 by both population and deep sequencing when compared with Trofile ES. As expected, the relative percentage of samples classified as non-R5 increased with higher FPRs; more viruses classified as R5 using lower FPRs were classified as non-R5.

Fewer patients had NR screening results using either genotypic method when compared with Trofile ES. Population genotypes were successfully determined for all 16 patients with an NR Trofile ES result at screening. Consistent with the composition of the overall population, as determined using genotyping and phenotyping, the subset of patients with NR Trofile ES results was found to contain 75% (12/16) R5 and 25% (4/16) non-R5 virus.

Samples classified as R5 by Trofile ES were compared with co-receptor usage predicted by the g2p algorithm using either population sequencing (5.75–10% FPR) or deep sequencing (3.5% FPR and 2% non-R5 threshold); concordance was in the range 83–92% (Fig. 1B). Concordance with population genotype decreased from 91.7% (at 5.75% FPR) to 83.3% (at 10% FPR); this is consistent with more viruses being classified as non-R5 at a higher FPR.

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