Short communication

Baseline susceptibility of primary HIV-2 to entry inhibitors

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Background: The baseline susceptibility of primary HIV-2 to maraviroc (MVC) and other entry inhibitors is currently unknown.
Methods: The susceptibility of 19 HIV-2 isolates obtained from asymptomatic and AIDS patients and seven HIV-1 clinical isolates to the fusion inhibitors enfuvirtide (ENF) and T-1249, and to the coreceptor antagonists AMD3100, TAK-779 and MVC, was measured using a TZM-bl cell-based assay. The 50% inhibitory concentration (IC_{50}), 90% inhibitory concentration (IC_{90}) and dose–response curve slopes were determined for each drug.
Results: ENF and T-1249 were significantly less active on HIV-2 than on HIV-1 (211- and 2-fold, respectively). AMD3100 and TAK-779 inhibited HIV-2 and HIV-1 CXCR4 tropic (X4) and CCR5 tropic (R5) variants with similar IC_{50} and IC_{90} values. MVC, however, inhibited the replication of R5 HIV-2 variants with significantly higher IC_{90} values (42.7 versus 9.7 nM; $P<0.0001$) and lower slope values (0.7 versus 1.3; $P<0.0001$) than HIV-1. HIV-2 R5 variants derived from AIDS patients were significantly less sensitive to MVC than variants from asymptomatic patients, this being inversely correlated with the absolute number of $CD4^+$ T-cells.
Conclusions: T-1249 is a potent inhibitor of HIV-2 replication indicating that new fusion inhibitors might be useful to treat HIV-2 infection. Coreceptor antagonists TAK-779 and AMD3100 are also potent inhibitors of HIV-2 replication. The reduced sensitivity of R5 variants to MVC, especially in severely immunodeficient patients, indicates that the treatment of HIV-2-infected patients with MVC might require higher dosages than those used in HIV-1 patients, and should be adjusted to the disease stage.

Introduction

HIV-2 affects an estimated 1–2 million individuals worldwide and leads to AIDS and death, albeit at a slower pace when compared to HIV-1. All currently available antiretroviral drugs were specifically designed to inhibit HIV-1 entry and replication. Consequently, some drug classes are not active on HIV-2 (that is, non-nucleoside reverse transcriptase and fusion inhibitors) and virological and immunological responses to treatment regimens incorporating active drugs are usually poorer in HIV-2 patients [1].

The envelope glycoproteins of HIV-1 and HIV-2 are markedly different at the amino acid sequence level and at the structural and functional levels. In contrast to HIV-1, HIV-2 may enter cells without binding to CD4, and by using multiple alternative coreceptors besides CCR5 and CXCR4 [2,3]. This suggests that maraviroc (MVC), a CCR5 antagonist, might also have limited activity against HIV-2. Currently, there is no information concerning the *in vitro* susceptibility of HIV-2 primary isolates to MVC,

enfuvirtide (ENF) or any other entry inhibitor. In the absence of formal clinical trials, *in vitro* evaluation of the baseline susceptibility of HIV-2 primary isolates to MVC is crucial to assess the potential clinical value of this drug in HIV-2 therapy [4]. Here we have analysed the susceptibility of HIV-2 primary isolates obtained from asymptomatic and AIDS patients to the fusion inhibitors ENF and T-1249 and to the coreceptor antagonists AMD3100, TAK-779 and MVC.

Methods

Primary isolates were obtained from HIV-2-infected Portuguese patients and, for comparison, from HIV-1-infected Angolan patients, all naive to therapy with entry inhibitors, by cocultivation with peripheral blood mononuclear cells from seronegative subjects (Table 1) [5]. Virus genotyping was performed by phylogenetic analysis using C2-V3-C3 (HIV-2) or gp41 (HIV-1) *env* sequences. GenBank accession numbers for newly derived sequences are HQ738345–HQ738350 for HIV-2 and HQ738338–HQ738344 for HIV-1.

CCR5 and CXCR4 tropism was determined using a single-round viral infectivity assay performed with TZM-bl reporter cells (CD4+, CCR5+ and CXCR4+) in the presence of excessive amounts of the CCR5 antagonist TAK-779 (10 µM) and/or of the CXCR4 antagonist AMD3100 (1.2 µM), as previously described [6]. The 50% inhibitory concentration (IC_{50}), 90% inhibitory concentration (IC_{90}) and dose–response curve slopes (Hill slopes) of ENF and T-1249 (fusion inhibitors) and AMD3100, TAK-779 and MVC (coreceptor antagonists) were determined on the newly derived panel of isolates (200× 50% tissue culture infective dose for each virus) using also the TZM-bl reporter cell assay. IC_{50}, IC_{90} and Hill slopes were estimated by the sigmoidal dose–response (variable slope) equation in Prism version 4.0c for Macintosh (GrahPad Software, San Diego, CA, USA). Prism was also used for statistical analyses (<u>*P*-value <0.05</u>).

Results

Genotypic and phenotypic characterization of virus isolates

A total of 19 new HIV-2 primary isolates were used in this study, all belonging to group A (Table 1 and Additional file 1). Overall, 10 were CCR5 tropic (R5 isolates), 8 CXCR4 tropic (X4 isolates) and 1 used both coreceptors (dual/mixed population). The seven new HIV-1 primary isolates were all R5 and their genotypes were distributed as follows: subtype G (1 isolate), J (2 isolates) and CRF02_AG (1 isolate); 3 isolates were untypable (Table 1 and Additional file 2).

Antiviral activity of coreceptor antagonists

AMD3100 and TAK-779 inhibited the replication of HIV-1 and HIV-2 with similar IC_{50}, IC_{90} and slope values (Tables 1 and 2). MVC also inhibited the replication of HIV-2 and HIV-1 R5 variants with similar IC_{50} values (Table 1); for HIV-1, the IC_{50s} were similar to previously reported values (range 0.1–4.5 nM; Table 1) [7]. However, MVC inhibited the replication of R5 HIV-2 variants with significantly higher IC_{90} values (42.7 versus 9.7 nM; *P*<0.0001) and lower slope values (0.7 versus 1.3; *P*<0.0001) than HIV-1 (Figure 1A and Table 2). R5 variants isolated from HIV-1 patients after AIDS diagnosis have reduced sensitivity to TAK-779 as compared to R5 variants isolated at the asymptomatic stage [8,9]. Strikingly, we also found a strong and significant negative correlation between HIV-2 sensitivity to MVC (as determined by the IC_{50s}) and CD4+ T-cell counts at the time of virus isolation (Spearman's *r*=-0.831; *P*=0.008; Figure 1B). Consistent with this, isolates from AIDS patients were significantly less sensitive to MVC (<u>that is, their IC_{50}s were significantly higher</u>) than isolates from asymptomatic patients (Figure 1C). A similar tendency was observed for TAK-779 (Additional file 3). In all, these results demonstrate that HIV-2 R5 variants have lower sensitivity to MVC than HIV-1 and suggest that resistance of these variants to MVC increases as disease progresses [8,9].

Antiviral activity of fusion inhibitors

In this study, ENF was 211-fold less active against primary isolates of HIV-2 than against HIV-1 (mean IC_{50} 281.5 versus 1.2 nM; *P*<0.0001; Figure 1D and Table 2), confirming and extending previous results based on lab adapted isolates [10]. Interestingly, with one exception, all HIV-1 primary isolates exhibited high sensitivity to ENF (Table 1). Sequencing analysis showed that these isolates carried the N42S polymorphism in the gp41 glycoprotein, whereas the less sensitive strain did not (data not shown). This polymorphism, which is more prevalent in several non-B subtypes and recombinant forms than in subtype B, has previously been associated with higher sensitivity to ENF both in B and non-B HIV-1 subtypes [11].

In contrast to ENF, T-1249 was active on HIV-2, although at higher concentrations than on HIV-1 (IC_{50} 4.3 versus 2.0 nM; *P*<0.0001). Moreover, T-1249 was more active on X4 than on R5 isolates both in HIV-1 (IC_{50} 0.6 versus 2.9 nM; *P*<0.0001) and HIV-2 (IC_{50} 3.2 versus 6.1 nM; *P*=0.0005).

Discussion

We have demonstrated that MVC inhibits the replication of R5 HIV-2 variants with significantly higher

Table 1. Clinical characterization of HIV patients and primary isolates including their susceptibility to different entry inhibitors

Isolates[b]	CD4+ T-cell count at study entry, cells/μl	HIV RNA, copies/ml	Antiretroviral therapy	Genetic forms[c]	Coreceptor use	Antiviral activity[a]									
						AMD3100, nM		TAK-775, nM		Maraviroc, nM		Enfuvirtide, nM		T-1249, nM	
						IC_{50}	IC_{90}	IC_{50}	IC_{90}	IC_{50}	IC_{90}	IC_{50}	IC_{90}	IC_{50}	IC_{90}
HIV-1															
93AOHDC249	NA	NA	No	U	R5	–	–	6.1	173.0	1.0	6.1	78.8	1,285.3	8.0	13.2
93AOHDC250	NA	NA	No	J	R5	–	–	8.1	7,516.2	2.4	9.7	0.4	15.7	1.9	8.7
93AOHDC251	NA	NA	No	U	R5	–	–	5.7	23,388.4	1.5	9.2	1.3	170.2	1.8	43.6
93AOHDC252	NA	NA	No	U	R5	–	–	153.5	24,434.3	4.7	48.5	3.7	23.5	2.6	8.0
93AOHDC253	NA	NA	No	J	R5	–	–	15.7	423.6	1.4	4.5	0.1	2.0	1.4	8.6
01PTHDECJN	1,003	<400	No	CRF02_AG	R5	–	–	178.4	5,942.9	2.7	11.9	0.7	132.1	1.6	6.7
00PTHDEEBB	409	2,742,788	No	G	R5	–	–	2.6	2,844.5	0.8	3.8	1.5	33.6	5.5	78.5
NL4-3	–	–	No	B	X4	0.9	6.0	–	–	–	–	5.0	178.6	0.6	1.0
SG3.1	–	–	No	B	X4	5.2	29.2	–	–	–	–	0.1	0.5	0.4	6.9
HIV-2															
03PTHCC1	308	<200	Yes	A	R5	–	–	0.6	1,219.0	0.9	4.8	35.6	877.0	5.1	49.3
03PTHCC6	615	<200	Yes	A	R5	–	–	10.1	2,301.4	0.9	32.3	661.1	2,192.8	8.4	74.1
03PTHCC7	144	<200	Yes	A	R5	–	–	16.2	3,581.0	2.9	28.9	549.1	2,138.0	7.3	12.6
03PTHCC12	66	<200	No	A	R5	–	–	45.1	7,030.7	3.8	78.7	2,857.0	32,062.7	6.3	36.6
03PTHCC17	367	<200	Yes	A	R5	–	–	3.0	55,080.8	0.9	27.9	138.4	2,162.7	2.5	40.3
03PTHCC19	175	NA	No	A	R5	–	–	128.3	167,880.4	4.3	81.5	250.0	1,729.8	7.2	24.6
00PTHDECT	2,919	1,355	No	A	R5	–	–	24.8	3,741.1	1.6	61.1	109.3	881.0	2.3	24.4
10PTHSJIG	164	4,257	Yes	A	R5	–	–	121.8	8,128.3	5.5	108.6	586.3	14,092.9	21.9	412.1
03PTHSM2	275	<200	Yes	A	R5	–	–	8.8	15,922.1	2.4	53.1	114.0	4,375.2	3.4	61.9
10PTHSMNC	231	<200	Yes	A	R5	–	–	57.4	3,396.3	2.2	40.1	265.4	3,507.5	8.9	71.3
10PTHSMAK	40	1,793	Yes	A	D/M	3.2	17.9	0.7	29,922.6	116.0	30,903.0	125.2	1,458.8	1.5	45.6
ROD	–	–	No	A	X4	1.0	16.1	–	–	–	–	76.1	3,380.6	9.1	174.6
03PTHCC10	48	<200	Yes	A	X4	3.6	78.3	–	–	–	–	293.6	3,047.9	2.4	12.4
00PTHCC20	1,033	<200	No	A	X4	1.9	17.5	–	–	–	–	151.3	1,422.3	0.9	8.4
03PTHCC20	78	<200	Yes	A	X4	2.0	18.6	–	–	–	–	362.7	3,548.1	1.9	10.9
03PTHDECT	209	20,968	No	A	X4	1.6	20.7	–	–	–	–	373.4	5,520.8	2.1	32.1
01PTHDESC	44	1,250	Yes	A	X4	4.0	32.7	–	–	–	–	241.5	3,672.8	4.9	49.1
03PTHSM9	15	<200	Yes	A	X4	4.2	27.7	–	–	–	–	1,281.0	6,729.8	7.0	12.6
04PTHSM10	265	4,792	Yes	A	X4	3.6	47.1	–	–	–	–	293.6	3,047.9	6.7	24.3
10PTHSMAUC	177	<200	No	A	X4	3.0	20.9	–	–	–	–	167.3	952.8	1.7	12.0

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[a]The 50% inhibitory concentration (IC_{50}) and 90% inhibitory concentration (IC_{90}) best-fit values were inferred from sigmoidal dose–response (variable slope) curves and represent geometric mean values of two independent experiments performed in duplicate wells; AMD3100 was only tested against CXCR4 tropic (X4) isolates, while TAK-779 and maraviroc were tested against CCR5 tropic (R5) isolates. [b]Lab-adapted reference strains NL4-3 (HIV-1), SG3.1 (HIV-1) and ROD (HIV-2) were obtained by transfection of 293T cells with pNL4-3 (HIV-1), pSG3.1 (HIV-1) or pROD10 (HIV-2) plasmids. [c]Untypable (U) HIV-1 isolates included 93AOHDC249 and 93AOHDC252 sequences, which are basal to subtypes 19_cpx and 37_cpx and the 93AOHDC251 sequence, which is basal to subtype H (Additional file 2). D/M, dual/mixed viral population using CCR5 and CXCR4 coreceptors; NA, not available.

Table 2. Comparison of antiviral activities of the different entry inhibitors on HIV-1 and HIV-2 primary isolates

Parameter[a]	HIV-1	HIV-2[b]	P-value[c]
AMD3100	–	–	–
Patients, *n*	2	9	–
IC_{50}, nM	2.1 (1.1, 3.8)	2.6 (2.2, 3.0)	0.288
IC_{90}, nM	16.7 (4.4, 62.8)	29.0 (20.8, 40.5)	0.213
Hill slope	1.0 (0.5, 1.6)	0.9 (0.8, 1.0)	0.391
TAK-779	–	–	–
Patients, *n*	7	10	–
IC_{50}, nM	23.3 (12.0, 45.4)	18.9 (11.8, 30.3)	0.595
IC_{90}, nM	5,200.0 (1,161.4, 23,334.6)	11,587.8 (3,899.4, 34,514.4)	0.379
Hill slope	0.4 (0.3, 0.5)	0.3 (0.3, 0.4)	0.237
Maraviroc	–	–	–
Patients, *n*	7	10	–
IC_{50}, nM	1.7 (1.4, 2.2)	2.1 (1.7, 2.6)	0.201
IC_{90}, nM	9.7 (6.6, 14.4)	42.7 (26.6, 68.4)	<0.0001
Hill slope	1.3 (1, 1.6)	0.7 (0.6, 0.8)	<0.0001
Enfuvirtide	–	–	–
Patients, *n*	9	20	–
IC_{50}, nM	1.2 (0.7, 2.2)	281.5 (223.2, 354.9)	<0.0001
IC_{90}, nM	95.9 (26.3, 350.8)	3,881.5 (2,393.3, 6,280.6)	<0.0001
Hill slope	0.5 (0.4, 0.6)	0.8 (0.7, 1)	0.001
T-1249	–	–	–
Patients, *n*	9	20	–
IC_{50}, nM	2.0 (1.4, 2.8)	4.3 (3.6, 5.2)	<0.0001
IC_{90}, nM	14.3 (6.9, 29.5)	40.6 (28.1, 58.5)	0.006
Hill slope	1.1 (0.8, 1.4)	1 (0.8, 1.1)	0.426

Data are mean (95% CI). [a]The 50% inhibitory concentration (IC_{50}), the 90% inhibitory concentration (IC_{90}) and slope best-fit values were inferred from sigmoidal dose-response (variable slope) curves adjusted to combined results of HIV-1 and HIV-2 isolates. [b]Estimates for AMD3100, TAK-779 and maraviroc did not include the HIV-2 10PTHSMAK isolate, a virus with dual/mixed tropism. [c]P-value for comparison of best-fit values between HIV-1 and HIV-2, using the F test.

IC_{90s} and lower slope values than HIV-1 indicating that higher dosages of MVC might be required for the treatment of HIV-2-infected patients [12,13]. So far, MVC use in HIV-2 infection was reported on only two occasions with uncertain results [14,15]. Clinical trials are therefore needed to determine if the MVC dosages recommended in HIV-1 infection are also effective for HIV-2 infection. This may prevent the administration of subtherapeutic dosages that favour the selection of X4 variants which, in HIV-2, have been associated not only with <u>CD4 depletion</u> and disease progression [2], but also with resistance to neutralization [16].
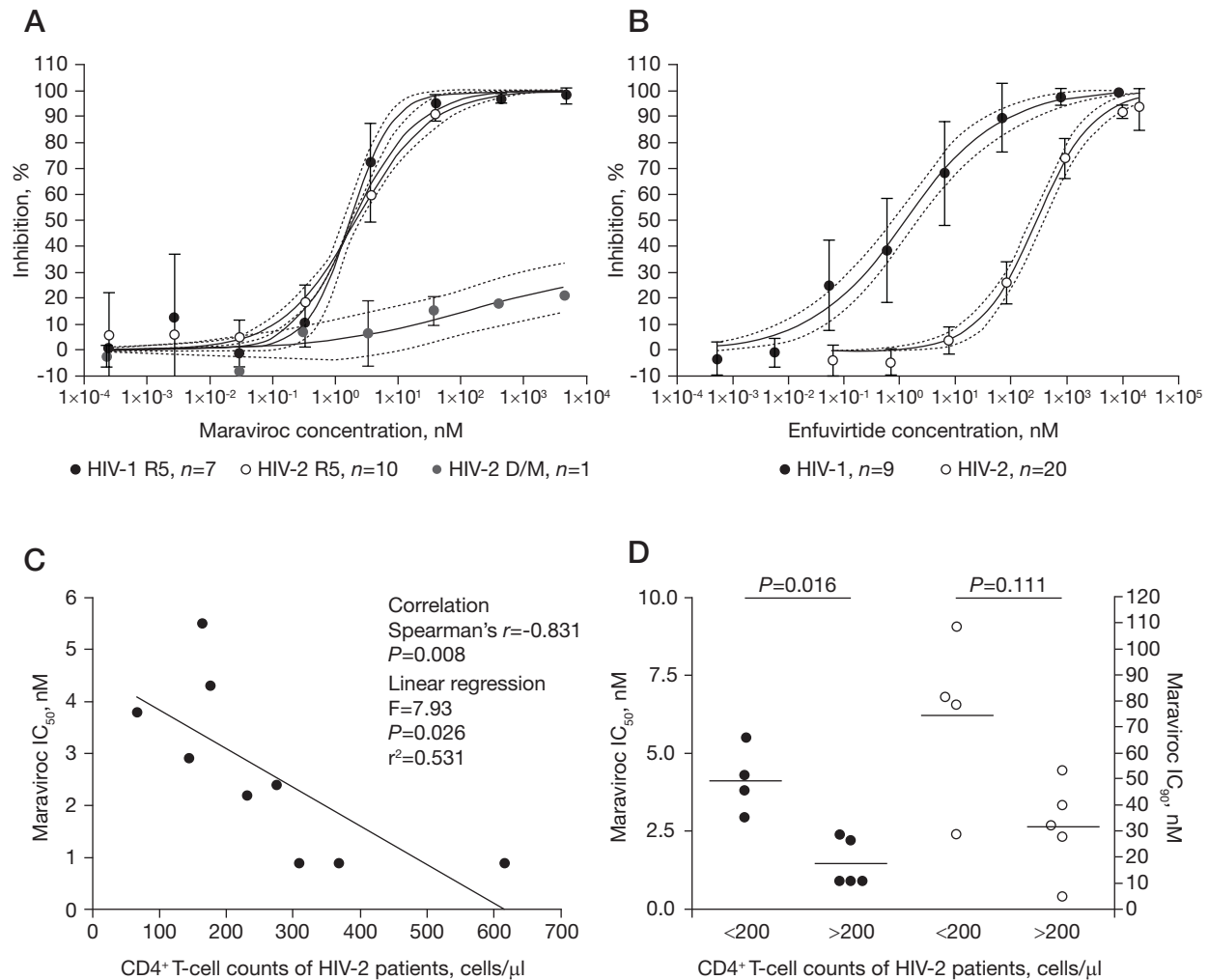
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Similarly to previous results obtained with RANTES for HIV-2 [17] and with TAK-779 and C-C chemokines for HIV-1 [8,9], MVC inhibits the replication of R5 HIV-2 variants isolated from AIDS patients with significantly higher IC_{50s} than R5 variants isolated from asymptomatic patients, this being inversely associated with the number of CD4+ T-cells. In HIV-2-infected patients, <u>CD4 depletion</u> and higher immune activation are also closely associated with an increased frequency of memory CD4+ T-cells expressing CCR5, the preferential target cells of this virus [18]. Hence, these results suggest that in HIV-2-infected patients MVC dosage

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may need to be adjusted according to the number of CD4+ T-cells (higher dosage in severely immunodeficient patients and lower dosage in asymptomatic patients). Increased MVC resistance of late stage disease R5 variants might be explained by increased affinity for CCR5 [19] and/or an enhanced viral infectivity and replicative capacity [8,19]. Alternatively, these R5 variants may be evolutionary intermediates toward X4 use [8,17].

The reduced activity of ENF on primary HIV-2 isolates provides definitive evidence that ENF is not useful for HIV-2 therapy. The low activity of ENF in HIV-2 is probably related to the high genetic variability between HIV-1 and HIV-2 in the HR1 and HR2 domains in the gp41 glycoprotein [10,20]. By contrast, T-1249, a second-generation fusion inhibitor available only for research use was highly active on both HIV-1 and HIV-2 indicating that new fusion inhibitors (peptides or small-molecules) might be useful to treat HIV-2 infection.

In summary, primary isolates of HIV-1 and HIV-2 with X4 or R5 tropism have similar sensitivities to AMD3100 and TAK-779, respectively. However, significantly higher IC_{90s} of MVC are required to inhibit

Figure 1. HIV-1 and HIV-2 susceptibility to clinically available fusion inhibitors

A Maraviroc concentration, nM; Inhibition, %
● HIV-1 R5, *n*=7   ○ HIV-2 R5, *n*=10   ● HIV-2 D/M, *n*=1

B Enfuvirtide concentration, nM; Inhibition, %
● HIV-1, *n*=9   ○ HIV-2, *n*=20

C Maraviroc IC$_{50}$, nM vs CD4$^+$ T-cell counts of HIV-2 patients, cells/μl
Correlation
Spearman's *r*=-0.831
P=0.008
Linear regression
F=7.93
P=0.026
r^2=0.531

D Maraviroc IC$_{50}$, nM / Maraviroc IC$_{90}$, nM vs CD4$^+$ T-cell counts of HIV-2 patients, cells/μl
P=0.016   *P*=0.111

Representative dose–response curves for HIV-1 and HIV-2 with **(A)** maraviroc and **(B)** enfuvirtide. Data points represent the mean of results obtained on HIV-1 and HIV-2 isolates and bars represent the 95% CI of the mean. Sigmoidal dose–response (variable slope) curves were adjusted to these data points; dashed lines represent the 95% confidence band of the best-fit curve. **(C)** Scatter plot of maraviroc 50% inhibitory concentrations (IC$_{50s}$) with the CD4$^+$ T-cell counts at the time of virus isolation in each HIV-2 patient infected with a CCR5 coreceptor tropism (R5) variant. Parameters from non-parametric correlation and linear regression analysis are shown. Isolate 00PTHDECT was excluded from this analysis since it was isolated from a child and therefore only CD4$^+$ T-cell percentage and not absolute CD4$^+$ T-cell counts should be considered. **(D)** Distribution of maraviroc IC$_{50}$ (closed circles) and 90% inhibitory concentration (IC$_{90}$; open circles) values according to two arbitrary levels of CD4$^+$ T-cells: <200 cells/μl, which is an AIDS-defining condition, and >200 cells/μl. Isolate 00PTHDECT was also excluded from this analysis. *P*-value for comparison of medians was determined using the non-parametric Mann–Whitney U test. D/M, dual/mixed viral population using CCR5 and CXCR4 coreceptors; X4, CXCR4 tropism.

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replication of HIV-2 R5 variants than HIV-1 variants. Additionally, the sensitivity of HIV-2 R5 variants to this drug is inversely related with CD4$^+$ T-cell counts at time of virus isolation. If MVC is to be used in HIV-2 patients, clinical trials should be performed to fully evaluate the clinical efficacy of this drug in HIV-2 infection and determine the best therapeutic dosage in early- and late-stage disease. Because X4 HIV-2 variants and dual/mixed HIV-2 populations are totally or partially resistant to MVC, coreceptor tropism should be determined before initiation of MVC therapy in HIV-2-infected patients. To this end, genotypic tropism assays, possibly based on the sequence of the V3 loop [2], should be developed to facilitate tropism assignment. Once used regularly in HIV-2 patients, the effect of MVC in the phenotypic evolution of this virus *in vivo* should be fully investigated as MVC has the potential to select for HIV-2 X4 variants that are associated with poor disease prognosis.

Acknowledgements

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Disclosure statement

The authors declare no competing interests.

Additional files

Additional file 1: Supplementary figure S1 illustrating genotyping of HIV-2 by maximum-likelihood phylogenetic analysis can be found at www.intmedpress.com/XXX

Additional file 2: Supplementary figure S2 illustrating genotyping of HIV-1 by maximum-likelihood phylogenetic analysis can be found at www.intmedpress.com/XXX

Additional file 3: Supplementary figure S3 illustrating the association between HIV-2 susceptibility to TAK-779 and the immunodeficiency degree of HIV-2 patients can be found at www.intmedpress.com/XXX

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