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Variable antiviral activity of islatravir against M184I/V mutant HIV-1 selected during antiretroviral therapy

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Background: Islatravir is a new antiretroviral drug that inhibits the reverse transcriptase (RT) of HIV-1 through multiple mechanisms. It is proposed to be used in combination with doravirine, a new NNRTI. M184V/I mutations have been shown to reduce the *in vitro* antiviral activity of islatravir, but their effect when pre-selected during ART has not been investigated.

Methods: HIV-1 *rt* sequences were obtained from four individuals of the Garrahan HIV cohort prior to, or during virological failure to ART. HIV-1 infectious molecular clones were constructed on an NL4-3 backbone, and infectious viruses were produced by transfection of 293T cells. Fold-changes in IC_{50} were calculated for each mutant versus the NL4-3 WT. HIV-1 phenotypic drug resistance was tested *in vitro* against NRTIs and NNRTIs.

Results: In all the cases, M184I/V, either alone or in the presence of other mutations, was associated with reduced susceptibility to islatravir, abacavir and lamivudine. Viruses carrying M184V/I showed variable levels of resistance to islatravir (4.8 to 33.8-fold). The greatest reduction in susceptibility was observed for viruses carrying the mutations M184V + V106I (33.8-fold resistance) or M184V + I142V (25.2-fold resistance). For NNRTIs, the presence of V106I alone did not affect susceptibility to doravirine or etravirine, but showed a modest reduction in susceptibility to efavirenz (6-fold). Susceptibility to doravirine was slightly reduced only for one of the mutants carrying V106I in combination with Y181C and M184V.

Conclusions: Mutations and polymorphisms selected *in vivo* together with M184V/I depend on the viral genetic context and on ART history, and could affect the efficacy of islatravir once available for use in the clinic.

Introduction

Islatravir or EFdA (formerly known as MK-8591) is a first-in-class investigational nucleoside reverse transcriptase translocation inhibitor (NRTTI) targeting the HIV-1 reverse transcriptase (RT). *In vitro*, islatravir has shown high activity against all subtypes with high potency (EC_{50} ~ 0.07 nM).[1] Because of its long half-life, islatravir is considered as a promising drug for long-term treatment.

In selection studies, islatravir has shown a high barrier to resistance, and a differentiated resistance profile from approved NRTIs.[2] Importantly, M184V or M184I were identified as the most frequent changes, and raised concern as to how these mutations could impact susceptibility to the new drug in ART-experienced populations where more than 50% of the population carry M184V/I due to prior exposure to emtricitabine or lamivudine. While these mutations typically cause more than 100-fold resistance to lamivudine or emtricitabine, the antiviral activity of islatravir for HIV-1 mutants carrying M184I/V as single mutations has been low or moderate (~2–10-fold).[3] However, selection of resistant viruses starting from a mixture of NRTI-resistant strains, instead of a single HIV-1 WT virus, resulted in a substantial level of resistance to islatravir of 100–150-fold, with M184V being the only evident mutation associated with islatravir resistance.[4,5] Other studies also testing the antiviral activity of islatravir against HIV-1 MDR molecular clones and clinical isolates showed up to 58.8-fold increases for M184V in combination with other RT mutations such as M41L, A114S, I142V, T165R and/or A400T.[2,6] Because selection of resistance to islatravir has not been determined *in vivo*, there is uncertainty as to how pre-existing M184I/V resistance mutations transmitted or selected during ART will affect the efficacy of this new drug once it is approved for use in the clinic.

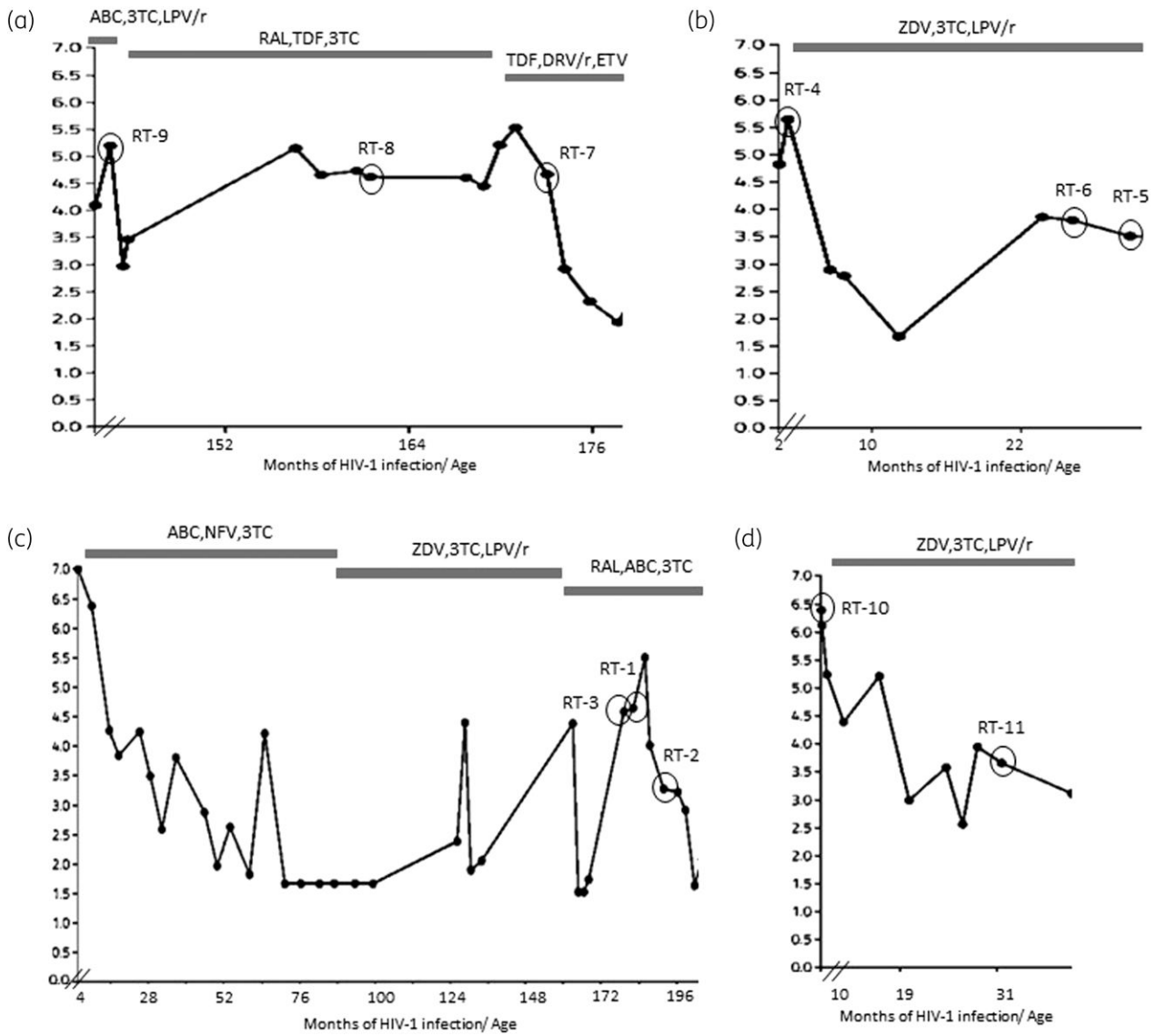

Figure 1. HIV-1 viral loads, ART regimens and timepoints evaluated for each of the cases from the study: (a) #6462, (b) #4675, (c) #3080 and (d) #5793. ABC, abacavir; 3TC, lamivudine; LPV/r, lopinavir/ritonavir; RAL, raltegravir; TDF, tenofovir disoproxil fumarate; DRV/r, darunavir/ritonavir; ETV, etravirine; NFV, nelfinavir; ZDV, zidovudine.

Islatravir is expected to potently inhibit viral replication in a long-acting formulation containing doravirine, a second-generation NNRTI approved in 2018 and currently indicated in combination with lamivudine and tenofovir for individuals without prior treatment failures or evidence of resistance to first-generation NNRTIs. The combination of islatravir + doravirine is currently being evaluated in Phase 2/3 clinical trials.[7] For doravirine, mutations associated with reduced susceptibility *in vitro* are similar to those observed *in vivo*, and include V106A/I, Y188L and F227C. Of them, V106I is a common polymorphism in some non-B subtypes, including BF recombinants common to Latin America.

To better understand the role of pre-acquired M184I/V mutations and V106I polymorphism on viral resistance to islatravir and doravirine, we studied viruses from four HIV-1 vertically infected patients with transmitted or acquired drug resistance, characterizing the *in vitro* phenotypic resistance on two to three mutant HIV-1 viruses representing the consensus viral populations isolated from the patients over time.

Materials and methods

HIV-1 *rt* sequences were selected from four patients belonging to the Garrahan HIV cohort, a well-characterized cohort of approximately 1500 children and adolescents with perinatal HIV-1 infection followed-up at 'Hospital de Pediatría J. P. Garrahan' in Argentina. HIV-1 viral load was determined with the COBAS Amplicor Roche v2 kit. HIV-1 genotyping was

Table 1. HIV-1 RT genotype and phenotype information of IMCs under study

Sample ID (patient ID#-virus ID)	Age/time of HIV-1 infection (months)	NRTI RAMs	NNRTI RAMs	Other mutations in RT	ISL IC_{50} FC	ABC IC_{50} FC	3TC IC_{50} FC	TFV IC_{50} FC	DOR IC_{50} FC	ETV IC_{50} FC	EFV IC_{50} FC
6462-RT9	147	M184V	Y181C	V35T,T39M,I135V,Q174K, I178M,V179I,G196E	4.8	1.9	>100	0.1	0.8	6.2	5.0
6462-RT8	161	K70Q, M184V	Y181C	E28A,V35T,T39M,T69N, I135V,I178M,V179I, G196E	9.4	2.6	>100	0.3	0.6	2.5	5.0
6462-RT7	173	K70S	Y181C	E28A,V35T,T39M,T69N, I135V,I178M,V179I, G196E	0.6	1.0	1.6	0.8	1.4	4.4	6.0
4675-RT4	2[a]	M184I	V106I, Y181C	I135V,S162C,F214L	13.9	1.6	>100	0.2	3.5	12.2	10.0
4675-RT6	26	M184I	V106I	V21I,I135V,S162C,F214L	21.9	3.0	>100	0.5	1.8	1.5	6.0
4675-RT5	30	M184V	V106I	V21I,I135V,S162C,F214L	33.8	1.4	>100	0.6	1.0	1.2	6.0
3080-RT3	178	M184V	—	K122E,D123E,I178M	14.1	3.3	>100	0.4	3.0	6.4	9.0
3080-RT1	181	M184V	—	K122E,D123E,I178M	9.6	2.2	>100	0.3	1.5	8.1	7.0
3080-RT2	190	M184V	—	K122E,D123E,I178M	14.0	2.6	>100	0.1	2.2	8.6	8.0
5793-RT10	10	—	—	V35T,K122E,I135V,I142V, K166R,R211K	0.8	0.8	1.2	0.7	1.0	1.4	3.0
5793-RT11	32	M184V	—	K13R,V35T,K122E,I135V, I142V,K166R,R211K	25.2	1.5	>100	0.4	0.7	1.5	6.0

ISL, islatravir; ABC, abacavir; 3TC, lamivudine; TFV, tenofovir; DOR, doravirine; ETV, etravirine; EFV, efavirenz. Mutations not associated with resistance that are not shared between viruses from the same patient are underlined. FC values were calculated setting IC_{50} of NL4-3 IC_{50}s for each drug at 1×: 2.7 ± 0.1 nM for ISL, 12.7 ± 1.9 μM for ABC, 1.3 ± 0.2 μM for 3TC, 12.6 ± 2.1μM for TFV, 2.8 ± 0.2 nM for DOR, 0.8 ± 0.2 nM for ETV and 0.1 ± 0.1 nM for EFV.
[a]Transmitted drug resistance.

performed upon virological failure using a clinically validated in-house method.[8]

Infectious molecular clones (IMCs) of HIV-1 bearing subtype F partial *pol* genes (codons 1–220 of RT) of BF recombinant viruses were generated using HIV-1_{NL4-3} (pNL4-3) as the backbone virus.[9] The predominant *rt* sequences (codons 1–220) identified in plasma samples were synthesized by GenScript, and inserted into HIV-1 NL4-3 by HiFi DNA Assembler (New England Biolabs). Constructs were confirmed by Sanger sequencing. Infectious virus stocks with each RT variant (RT1–RT11) were generated by transfection, infectious titres determined, and samples stored at −80°C.

Phenotypic susceptibility analyses of RT inhibitors were performed with recombinant RT viruses in TZM-bl (JC53) cells contributed by Dr J. C. Kappes and Dr X. Wu as previously described.[10,11] Susceptibility of the viruses to antiretrovirals was examined using 10-fold serial dilutions. Concentration ranges were 1000 to 10^{-3} nM for islatravir, 10000 to 10^{-2} nM for doravirine (both purchased from MedChemExpress), 1000 to 10^{-3} nM for etravirine and efavirenz, 1000 to 10^{-3} μM for abacavir and lamivudine, and 150 to 1.5×10^{-4} μM for tenofovir. The IC_{50} was calculated by non-linear regression using Prism 9 (GraphPad Software).

Results

Phenotypic drug resistance to RT inhibitors was determined on 11 HIV-1 molecular clones from four different HIV-1 vertically infected patients. Selection of cases was based on the presence of mutations at codon 184 of HIV-1 RT (M184V/I) in at least one of the viruses recovered during infection. Two patients (#5793 and #4675) were studied prior to ART, and during their first virological failure. The remaining two patients (#3080 and #6462) were studied at three different timepoints under non-suppressive ART regimens. Information about HIV-1 viral loads, ART regimens and timepoints evaluated for each of the cases is shown in Figure 1.

For reference virus NL4-3, islatravir demonstrated potent activity with an IC_{50} of 2.7 nM. This was approximately 5000-fold higher than the potency of abacavir and tenofovir, and 1000-fold higher than that of lamivudine. As shown in Table 1 and Table S1 (available as Supplementary data at *JAC* Online), viruses carrying M184V or M184I mutations showed reduced susceptibility to islatravir, with IC_{50}s between 12.9 and 91.2 nM, representing a fold-change (FC) range of 4.8 to 33.8. The greatest reduction in susceptibility was observed for patient #4675 carrying mutations M184I or M184V + V106I in three viruses recovered at 2, 26 and 30 months of age (RT4, RT6 and RT5, respectively). The combination M184V + V106I present in RT5 was associated with the highest level of resistance in our study (33.8-fold). Of note, a 25.2-fold resistance was also observed in virus RT11 recovered during virological failure at 32 months of age from patient #5793. In this case, the presence of the drug-associated mutation M184V was accompanied only by RT polymorphisms K13R, V35T, K122E, I135V, I142V, K166R and R211K. As expected, M184V/I mutations drastically reduced susceptibility to lamivudine in all cases, and each of the viruses maintained full susceptibility to abacavir and tenofovir, in agreement with a lack of drug-resistance associated mutations for these antiretrovirals.

The effect of V106I on drug susceptibility was assessed in viruses from case #4675 (RT4 to RT6). Presence of V106I alone did not seem to affect susceptibility to doravirine or etravirine, but modestly reduced susceptibility to efavirenz. Only the combination of V106I+Y181C and M184V present in RT4 resulted in a 3.5-fold resistance to doravirine.

Discussion

Using the complete 5′ portion of *rt* of four individuals infected with HIV-1 at birth and followed over 30 to 190 months, in our study we found that M184I/V mutations in combination with others, either present as polymorphisms or previously selected *in vivo* during ART, could result in islatravir IC_{50} increases as high as 33.8-fold.

The highest resistance to islatravir occurred in a virus carrying M184V in combination with V106I, a doravirine-associated mutation that is usually found as a polymorphism in BF recombinant strains circulating in South America, reinforcing the need to comprehensively evaluate HIV-1 drug resistance in the context of HIV-1 genetic diversity. An important reduction in susceptibility to islatravir was also observed in a patient carrying I142V before initiation of ART, and selecting M184V as a single mutation in this context. I142V has not previously been associated with drug resistance to antiretrovirals, and occurs at a very low frequency as a polymorphism. However, our result is in agreement with at least one previous study reporting that the addition of I142V and/or T165R augments the effect of M184V resistance to this compound, with a concomitant loss of viral fitness.[6] While the presence of one or more mutations or polymorphisms could contribute to the observed phenotypes in our study, we also found differences in FC between viruses with almost no *in vivo* evolution and identical in amino acid sequences. Thus, the contribution of other mutations to the loss of susceptibility conferred by M184I/V mutations will need to be further explored.

For doravirine, we found little or no effect of V106I polymorphism on drug susceptibility in the context of BF recombinant *rt* genes. However, this polymorphism can augment or facilitate selection of additional resistance mutations, as recently observed in subtype B strains *in vitro*[12], and will therefore merit further research.

Viruses carrying M184V or M184I mutations showed reduced susceptibility to islatravir, with IC_{50}s between 12.9 and 91.2 nM, representing an FC range of 4.8 to 33.8, indicating the potential for escape. While the barrier to resistance is thought to be high, the lack of *in vivo* resistance evolution in response to islatravir hampers understanding of the significance of its *in vitro* phenotypic resistance.

Pre-existing mutations in the HIV-1 target genes may compromise the efficacy of the new antiretroviral drugs. Identifying mutations that confer resistance to them is important for an accurate prediction of HIV-1 drug susceptibility based on genotypic-based scores, and to guarantee maximal antiviral response based on genotyping.

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Transparency declarations

None to declare.

Supplementary data

Table S1 is available as Supplementary data at *JAC* Online.

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