

New raltegravir resistance pathways induce broad cross-resistance to all currently used integrase inhibitors

Isabelle Malet^{1–3*}, Laura Gimferrer Arriaga^{1–3}, Anna Artese⁴, Giosuè Costa⁴, Lucia Parrotta⁴, Stefano Alcaro⁴, Olivier Delelis⁵, Ahmed Tmeizeh^{1–3}, Christine Katlama^{1–3}, Marc-Antoine Valantin^{1–3}, Francesca Ceccherini-Silberstein⁶, Vincent Calvez^{1–3} and Anne-Geneviève Marcelin^{1–3}

¹Sorbonne Universités, UPMC Univ Paris 06, UMR_S 1136, Institut Pierre Louis d'Epidémiologie et de Santé Publique, F-75013, Paris, France; ²INSERM, UMR_S 1136, Institut Pierre Louis d'Epidémiologie et de Santé Publique, F-75013, Paris, France; ³AP-HP, Laboratoire de Virologie, Hôpital Pité-Salpêtrière, F-75013, Paris, France; ⁴Dipartimento di Scienze della Salute, Università degli Studi 'Magna Græcia', Campus 'S. Venuta', Viale Europa, Germaneto, Catanzaro 88100, Italy; ⁵LBPA, CNRS, Ecole Normale Supérieure de Cachan, 94235 Cachan, France; ⁶Department of Experimental Medicine, University of Rome Tor Vergata, Rome, Italy

*Corresponding author. Tel: +33-142177401; Fax: +33-142177411; E-mail: isabelle.malet@ppl.aphp.fr

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Objectives: The possibility of replacing raltegravir or elvitegravir with dolutegravir in heavily treatment-experienced patients failing on raltegravir/elvitegravir has been evaluated in VIKING trials. All studied patients failed by the most common pathways, Y143, Q148 and N155, and dolutegravir demonstrated efficacy except for Q148 viruses. The aim of this study was to explore, in the same way, the behaviour of dolutegravir in comparison with raltegravir and elvitegravir against the atypical resistance integrase profiles, G118R and F121Y, described in HIV-1 patients failing on raltegravir therapy.

Methods: The behaviour of integrases with mutations G118R and F121Y towards raltegravir, elvitegravir and dolutegravir was analysed by evaluating phenotypic susceptibility and by means of *in silico* techniques (investigating binding affinities and the stabilization of the inhibitors in terms of their hydrogen bond network).

Results: The phenotypic analysis of G118R and F121Y showed high resistance to raltegravir, elvitegravir and dolutegravir with a fold change >100 when the clinically derived integrase was used, and resistance was also seen when mutations were tested alone in an NL43 backbone, but more often with a lower fold change. *In silico*, results showed that G118R and F121Y enzymes were associated with reduced binding affinities to each of the inhibitors and with a decreased number of hydrogen bonds compared with the wild-type complexes.

Conclusions: This study showed that G118R and F121Y mutations, rarely described in patients failing on raltegravir, induced broad cross-resistance to all currently used integrase inhibitors. These results are in accordance with our thermodynamic and geometric analysis indicating decreased stability compared with the wild-type complexes.

Keywords: dolutegravir, raltegravir, elvitegravir, phenotype, binding affinity

Introduction

Integrase inhibitors represent a novel class of drugs completely active against viruses resistant to all other classes of drugs. Raltegravir was the first inhibitor approved by the US FDA, in 2007, for HIV-1 treatment. Recently, the class of integrase inhibitors was expanded with two new approved inhibitors, elvitegravir (GS-9137, Gilead), used as co-formulated elvitegravir/cobicistat/tenofovir/emtricitabine, and dolutegravir (S/GSK 1349572, GlaxoSmithKline), a second-generation inhibitor. Raltegravir and elvitegravir share a common clinical resistance profile, including primary resistance mutations, often in combination with secondary mutations that

further compensate for the decreased fitness associated with the primary mutations. Two major resistance pathways are Q148HRQ/G140S and N155H/E92Q with a third, less frequent, pathway, Y143CRH/T97A, specifically described for raltegravir and specific resistance mutations, T66I and S147G, only described for elvitegravir.^{1–5} *In vitro* studies showed that dolutegravir has a resistance profile markedly distinct from those of raltegravir and elvitegravir. Several mutations involving non-polymorphic residues, such as S153YF, R263K and G118R, have been described as being selected by dolutegravir.^{6,7} The possibility of replacing raltegravir/elvitegravir with dolutegravir in heavily treatment-experienced patients failing on raltegravir or elvitegravir with viruses harbouring

resistance integrase mutations in the Y143, Q148 and N155 pathways was evaluated in VIKING trials. Dolutegravir demonstrated efficacy against most isolates resistant to raltegravir and elvitegravir except for viruses containing mutations of the Q148HKR pathway, with at least one mutation among G140ACS, L74I and E138AKT that conferred lower susceptibility to dolutegravir.⁸

In this report, we evaluated the phenotypic susceptibility to all currently used integrase inhibitors (raltegravir, elvitegravir, dolutegravir) of two atypical resistance profiles, G118R and F121Y, described in two HIV-1-infected patients failing on raltegravir therapy. By means of *in silico* techniques, we predicted integrase inhibitor binding affinities towards the wild-type and the two mutated integrase complexes and evaluated the stabilization of the inhibitors in terms of their hydrogen bond network.

Methods

We studied two patients failing on raltegravir therapy with atypical resistance profiles. Patient 1 was infected with a CRF02_AG virus and was treated with abacavir, lamivudine and raltegravir. At failure, at month 17, viral load was 1900 copies/mL and the G118R mutation was detected. Patient 2 was infected with a subtype B HIV-1 strain and was treated with maraviroc and raltegravir. We showed at the time of failure, after 22 weeks of treatment, an HIV-1 plasma viral load of 2820 copies/mL and the appearance of the F121Y mutation.

To assess phenotypic susceptibility, we first constructed clones containing the two individual integrase substitutions, G118R and F121Y, by site-directed mutagenesis in pNL43 containing the complete HIV-1 genome by using the QuikChange II XL Site-Directed Mutagenesis kit (Stratagene). Secondly, we generated clones with clinically derived integrase coding regions synthesized by Proteogenix by using a recombination procedure with the CloneEZ PCR cloning kit (GenScript). Briefly, to assess

phenotypic susceptibility, replication-competent recombinant viruses were titrated and subjected to an antiviral experiment in serial dilutions of raltegravir, elvitegravir and dolutegravir using HeLa CD4 LTR-LacZ cells. Fold changes in IC₅₀ values were calculated.²

Since it has proven difficult to crystallize full-length HIV integrase and its complexes with DNA and inhibitors, experimental structures derived from a related retrovirus, the prototype foamy virus (PFV), are available in the Protein Data Bank (PDB). However, in a recent study, Barreca *et al.*⁹ built a 3D model of a complex between HIV-1 integrase, viral DNA and metal ions, used as a target to evaluate the molecular recognition of raltegravir by an induced-fit docking (IFD) method.¹⁰ Starting from this model, we decided to apply the IFD approach and evaluated the molecular recognition of the three integrase inhibitors. Initially, both ligands and enzyme were pretreated using the Maestro Build Panel.¹¹ An initial Glide SP docking of each ligand was carried out, and 20 poses per ligand were energy minimized with the OPLS-AA force field.¹² After Prime minimization of the residues and the ligand for each pose, a Glide SP redocking of each protein–ligand complex structure was performed. Finally, each output pose of raltegravir, elvitegravir and dolutegravir was used as the starting structure for the Liaison prediction model¹³ for wild-type and mutated complexes. In particular, we adopted the truncated Newton algorithm to perform 1000 minimization steps, using a residue-based cut-off distance equal to 15 Å and OPLS2005¹⁴ as force field with the all atoms notation. Finally, all the wild-type and mutated integrase complexes were evaluated in terms of hydrogen bonds using the Maestro graphical interface¹¹ and the results are included in Table 1.

Results

The genotypic analysis for Patient 1 at baseline showed the presence of several residues previously described as specific for the CRF02_AG subtype,¹⁵ but also the presence of the 72I and 74M

Table 1. Behaviour of the integrase inhibitors raltegravir, elvitegravir and dolutegravir towards wild-type integrase and the two mutated integrases G118R and F121Y, by phenotypic analysis and in terms of binding affinities and stabilization of all complexes

Genotype	Integrase tested	Phenotypic analysis			Binding affinities of integrase inhibitors to the integrase ^a						Number of hydrogen bonds established by integrase inhibitors with integrase and DNA ^b		
		fold change ^c			van der Waals (kcal/mol)			electrostatic energy (kcal/mol)			DTG	RAL	EVG
		DTG	RAL	EVG	DTG	RAL	EVG	DTG	RAL	EVG			
WT	wild-type	—	—	—	−480.83	−473.75	−436.83	−1339.85	−1518.33	−1063.20	3	3	1
G118R	clinically derived integrase SDM	>100 10	>100 8.2	>100 3.1	−404.84	−426.56	−385.13	−238.37	−785.65	−744.78	0	1	0
F121Y	clinically derived integrase SDM	>100 5	>100 >100	>100 >100	−372.71	−424.67	−399.32	−718.32	−1364.38	−776.89	1	0	1

DTG, dolutegravir; RAL, raltegravir; EVG, elvitegravir; SDM, site-directed mutagenesis; WT, wild-type.

^aThe single average contributions of van der Waals and electrostatic energy components were evaluated between integrase inhibitors and HIV-1 integrase as binding affinities.

^bThe numbers of hydrogen bonds between integrase inhibitors and HIV-1 integrase residues and DNA were established using the IFD approach.

^cValues are given as average fold changes compared with the NL43 wild-type HIV-1.

integrase residues, previously described as linked to raltegravir resistance and the appearance of G118R at raltegravir failure. Patient 2 showed at baseline some residues described as polymorphic and then, at raltegravir failure, the appearance of F121Y. The identification of these two atypical integrase mutations at failure of raltegravir led us to assess phenotypic susceptibility to dolutegravir compared with raltegravir and elvitegravir. The phenotypic analysis showed high resistance to raltegravir, but also to elvitegravir and dolutegravir, with a fold change >100 compared with NL43 wild-type, when the clinically derived integrase was evaluated (Table 1). However, when mutations were tested alone in an NL43 backbone, we found some differences with the F121Y mutation, which showed low resistance to dolutegravir with a 5-fold change, compared with high resistance

to raltegravir and elvitegravir (fold change >100). Lower fold changes were measured with the G118R mutation: 3.1 for elvitegravir and ~10 for raltegravir and dolutegravir (Table 1).

Our computational results supported the experimental observations. In particular, the integrase inhibitor molecular recognition analysis revealed that all the inhibitors were associated with reduced binding affinities for G118R and F121Y mutated enzymes compared with the wild-type complexes, thus indicating decreased stability according to their high resistance profile.

Analysing all drug–integrase complexes, we observed that the three inhibitors were less stably recognized into the integrase binding pocket in the mutated enzymes, as shown by their unfavourable van der Waals and electrostatic energy terms with respect to the wild-type sequence. Specifically, as reported in

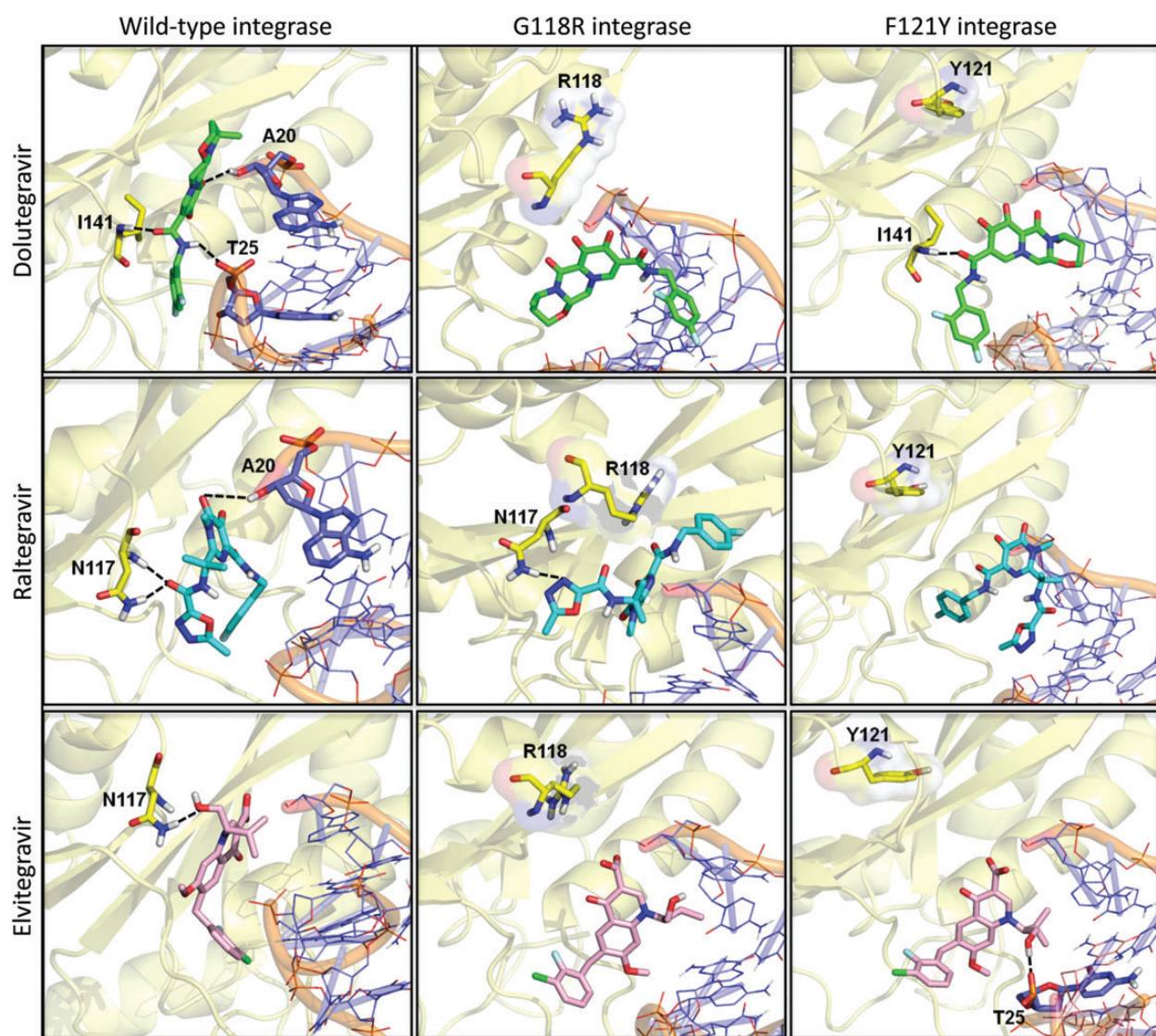


Figure 1. Best configurations view of dolutegravir (green carbon sticks), raltegravir (cyan carbon sticks) and elvitegravir (pink carbon sticks) towards the wild-type, G118R and F121Y integrase complexes. The enzyme is shown as a pale yellow transparent cartoon and the DNA as an orange cartoon with the nucleobases in slate wireframe. The integrase residues involved in hydrogen bonds with the inhibitor are represented as yellow carbon sticks and the contacts are indicated with a black dashed line. The mutated amino acids are shown as yellow carbon sticks with a transparent surface. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

Table 1, the G118R substitution was always associated with a markedly reduced electrostatic contribution compared with wild-type. This observation is probably due to strong electrostatic contacts established by the arginine at position 118 with the integrase acidic residues D64, E92 and D116, able to attract and distancing it from the inhibitor.

Our thermodynamic analysis was further supported by the geometric evaluation of all best configurations of integrase inhibitors. As reported in Table 1 and Figure 1, in the raltegravir–wild-type complex the drug was involved in two hydrogen bonds with N117 and another one with the DNA; by contrast, in the presence of the G118R mutation the inhibitor was able to maintain only one of the hydrogen bonds with N117, while completely missing all its contacts in the F121Y mutated enzyme (Figure 1). In the wild-type model, the asparagine at position 117 was also crucial in elvitegravir stabilization with one hydrogen bond (Figure 1); in the mutated complexes the drug loses such a contact (Figure 1), according to its thermodynamic profile. Interestingly, in contrast with the raltegravir and elvitegravir observations, the isoleucine at position 141 was found to play a pivotal role in dolutegravir binding. Specifically, as shown in Figure 1, in the wild-type complex the drug was well stabilized by one hydrogen bond with I141 and two hydrogen bonds with the nucleic acid. Only in the F121Y-mutated enzyme was dolutegravir found to maintain productive interaction with the amino acid, while fully abrogating all the contacts in the G118R complex (Figure 1).

Discussion

We studied two atypical HIV integrase G118R and F121Y substitutions appearing in two patients who failed raltegravir therapy, in subtype CRF02_AG and B viruses, respectively. G118R was selected *in vitro* in the presence of the experimental integrase inhibitor MK-2048 in C virus⁶ and more recently with dolutegravir in C and CRF02_AG viruses.¹⁶ This mutation has also been selected in a patient failing on raltegravir therapy and harbouring a subtype CRF02_AG virus.¹⁷ F121Y, first selected *in vitro* by raltegravir and elvitegravir,^{5,18} has also been reported recently in Brazil, as selected in a subtype B-infected patient failing on raltegravir alongside other mutations—L74I, T97A, Q137H and V151I.¹⁹ The results of this study showed that the two non-polymorphic mutations G118R and F121Y induced broad cross-resistance to all currently used integrase inhibitors. In most cases, higher increases in terms of fold change were found with clinical isolates compared with site-directed mutagenesis with an NL43 backbone. These differences could probably be explained by the presence of polymorphic residues in clinical isolates. For example, in the case of G118R, L74M could have a potential impact as this mutation has already been described in raltegravir failure and was also selected by dolutegravir in patients previously treated with raltegravir.²⁰

Results are in accordance with our thermodynamic and geometric analysis, which highlighted that all integrase inhibitors are associated with reduced binding affinity and a decreased hydrogen bond network in the presence of G118R and F121Y mutations compared with the wild-type sequence, which is consistent with the experimental observations. Even though these profiles are infrequent at the moment, they need to be monitored in all current patients treated with integrase inhibitors.

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Transparency declarations

None to declare.

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