Mutations Associated with Failure of Raltegravir

Treatment affect integrase sensitivity to the inhibitor in vitro

Running title: resistance to raltegravir

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Raltegravir (MK-0518) is a potent inhibitor of HIV integrase and is clinically effective against viruses resistant to other classes of antiretroviral agents. However, it can select mutations in the HIV integrase gene. Nine heavily pretreated patients who received a salvage therapy including raltegravir and who subsequently developed virological failure under raltegravir therapy were studied. For each patient, sequences of integrase-coding region were determined and compared to that at the beginning of the treatment. Four different mutation profiles were identified in these nine patients: E92Q, G140S + Q148H, N155H and E157Q mutations. For four patients, each harboring a different profile, the wild-type and mutated integrases were produced, purified and assayed in vitro. All the identified mutations altered the activities of integrase protein: E92Q was moderately affected for both 3’ processing and strand transfer activities; N155H was markedly impaired for strand transfer; G140S + Q148H was strongly impaired for both activities; and E157Q was almost completely inactive for both activities. The sensitivities of wild-type and mutant integrases to raltegravir were compared. The profiles E92Q and G140S + Q148H were each associated with a 7- to 8-fold decrease in sensitivity, and N155H was more than 14-fold less sensitive to raltegravir. At least four genetic profiles (E92Q, G140S + Q148H, N155H and E157Q) can be associated with in vivo treatment failure and
resistance to raltegravir. These mutations led to enzymes strongly impaired in vitro in the absence of raltegravir, strand transfer activity was affected and in some cases the 3’ processing was also impaired.
INTRODUCTION

The retroviral integrase (IN), a pol gene product, is responsible for the integration of the retroviral DNA into the host cell genome. Integration is an essential step of HIV-1 replication.

HIV-1 IN (32 kDa) is a 288 amino acid protein (aa) and consists of three independent structural domains (8, 18, 21). The N-terminal domain (aa 1-49) contains a HHCC motif, which binds zinc, thereby promoting multimerization to tetramers (6, 26, 27), a protein state required for strand transfer activity (6, 26, 27); the central catalytic core domain (aa 50-212) contains the D, D(35)E catalytic motif (Asp64, Asp116 and Glu152), in a highly conserved spatial arrangement (22); and the C-terminal domain (aa 213-288) has DNA-binding activity (3, 28).

IN catalyses two reactions. The first is 3’-end processing during which the terminal GpT dinucleotide is cleaved from the 3’ end of each LTR, producing CpA 3’-hydroxyl ends (9). This reaction takes place in the cytoplasm within a nucleoprotein complex, referred to as the preintegration complex (PIC) (30). The PIC is transported through the nuclear pore to the nucleus where the second activity — strand transfer — occurs. During this second step, IN transfers both newly exposed 3’extremities of the viral DNA into the target DNA by a one-step transesterification, the viral genome is thereby inserted and covalently linked...
Integrase inhibitors, a new class of antiretroviral agents, block HIV-1 integrase activity (25, 33). Several classes of inhibitors interfering either with the 3'-end processing in the cytoplasm (1, 32) or the strand transfer in the nucleus (10, 11, 13, 15, 38) have been described. Although both classes were proven to be able to block HIV replication in cell culture (1, 15), only the second class was found to possess an antiviral activity in vivo (5, 16, 29, 34). Due to its mechanism of action, this novel class of antiretroviral agent (ARV) is potentially valuable as it is active against viruses resistant to other classes of antiretrovirals such as NRTI, NNRTI, PI and entry inhibitors. Raltegravir (MK-0518) is a member of this class of novel HIV-1 inhibitors interfering with the strand transfer stage. It is active in patients infected with drug-resistant viruses when used in combination with an optimized regimen: an approximately 2 log decrease in HIV RNA is observed by week 24. However, it has been suggested that viral failure in patients under treatment with this compound is due to mutations in the integrase gene (D. Cooper, J. Gatell, J. Rockstroh, C. Katlama, P. Yeni, A. Lazzarin, J. Chen, R. Isaacs, H. Teppler, BY. Nguyen, for the BENCHMRK-1 study group, Abstr. 14th Conf. Retrovir. Opport. Infect., abstr. 105aLB, 2007 and R. Steigbigel, P. Kumar, J. Eron, M. Schechter, M. Markowitz, M. Loutfy, J. Zhao, R. Isaacs, BY. Nguyen, H. Teppler, for the BENCHMRK-2 study group, Abstr. 14th Conf. Retrovir. Opport. Infect., abstr.
Here, we describe the genetic changes in the integrase gene in nine heavily pretreated patients who received a salvage therapy including raltegravir and who subsequently suffered virological failure. This study identified four different profiles. The catalytic activities of the corresponding wild-type (pre-salvage treatment) and mutated recombinant integrases derived from four patients, each one harboring a different genetic change in the IN gene, were assayed in vitro and their sensitivities to raltegravir were compared.
MATERIAL AND METHODS

Study design and subjects. Nine patients receiving raltegravir as part of an expanded access program in France and who suffered virological failure while on this regimen were studied. The genotypic analysis of resistance of IN was performed following the French National Guidelines for the use of resistance testing (www.hivfrenchresistance.org). All these patients received at least one NRTI and one boosted PI associated +/- enfuvirtide in their optimized regimen. The optimized regimen associated with raltegravir was selected according to previous antiretroviral exposure and genotypic resistance testing interpreted with the French ANRS AC11 algorithm v16 (www.hivfrenchresistance.org).

RNA isolation, cDNA synthesis and PCR. Plasma (500 µl) was centrifuged at 19,000 x g for 1h at 4°C and viral RNA was extracted from the pellet using the Cobas Amplicor Test (Roche). Ten microliter aliquots of RNA were used for reverse transcription (RT)-PCR (Titan One-Tube RT-PCR kit; Roche Applied Science) following the manufacturer’s instructions and using 0.4 µM (final concentration) of each primer (IN12 and IN13) located in the integrase gene (17). The RT step was carried out at 50°C for 30 min and PCR involved 40 repeat cycles (94°C for 30 s, 56°C for 30 s, and 68°C for 1 min)
and then incubation at 68°C for 7 min. The second-round PCR using the AmpliTaq DNA polymerase (Applied Biosystems) was performed following the manufacturer’s instructions and using 1 μM (final concentration) of each primers IN1 and BH4 (17). PCR involved 40 repeat cycles (94°C for 1 min, 55°C for 1 min, and 72°C for 1 min) and then incubation at 72°C for 7 min. The PCR product was purified with the Amicon Microcon-100 (Millipore) for sequencing or purified on agarose gel for cloning.

Sequencing. The integrase gene was sequenced using a cycle sequencing reaction with the Big Dye terminator kit (Applied Biosystems). A set of four primers was used for complete coverage of both strands of the integrase gene. The primer sequences were as follows, with their positions in the HIV-1 HxB2 sequence indicated in brackets: two forward primers (nt 4137 to 4157) IN1 (17), (nt 4542 to 4558) IN4542S 5’-GCAGGAAGATGGCCAGT-3’ and two reverse primers (nt 4743 to 4764) IN4764AS 5’-CCATTTGTACTGCTGTCTTAA-3’ and (nt 5200 to 5222) BH4 (17). The sequences were analyzed using Sequence Navigator software.

Production and purification of integrase. The PCR products corresponding to the entire IN sequences were digested with Ndel and BamHI
and ligated into pET-15b, a bacterial expression vector (Novagen). The IN
sequence was verified by sequencing for all constructs.

His-tagged INs were produced in *Escherichia coli* BL21 (DE3) and purified
under non-denaturing conditions as previously described (27). Protein
production was induced at an OD of 0.7 by addition of IPTG to 1 mM. Cultures
were incubated for 3 h at 37°C, then centrifuged. The cells were resuspended in
buffer A (50 mM Tris-HCl (pH8), 1 M NaCl, 4 mM β-mercaptoethanol) and
lysed with a French Press. The lysate was centrifuged (30 min at 10,000 rpm)
and the supernatant was filtered (0.45μM) and incubated over-night with Ni-
NTA agarose beads (Qiagen). The beads were washed with buffer A and then
extensively with buffer A supplemented with 80mM imidazole. His-tagged
proteins were then eluted from the beads with buffer A supplemented with 1M
imidazole and 50μM zinc sulfate. They were then dialysed overnight against 20
mM Tris-HCl (pH8), 1 M NaCl, 4 mM β-mercaptoethanol and 10% glycerol.
The samples were aliquoted and rapidly frozen at -80°C.

**IN activity assay.** For activity assays, the 21-mer ODN U5 (5’-
GTGTGGAAAATCTCTAGCAGT-3’) was radiolabeled with T4
polynucleotide kinase (Biolabs) and γ[32-P]ATP (3000 Ci/mmol) (Amersham),
and purified on a Sephadex G-10 column (GE Healthcare). Double-stranded
ODN substrate was obtained by mixing equimolar amounts of labeled U5B and
its complementary strand (5'-ACTGCTAGAGATTTCACAC-3') in the presence of 100 mM NaCl. IN assays were carried out for 1 hr at 37°C, in a buffer containing 10 mM HEPES (pH7.2), 1 mM DTT, 7.5 mM magnesium chloride in the presence of 12.5 nM dsDNA substrate and 200nM recombinant IN. Products were separated by electrophoresis in denaturing 18% acrylamide/urea gels. Gels were analyzed with STORM 840TM phosphoimager (GE Healthcare Life sciences, Piscataway, USA) and quantified with ImageQuantTM 4.1 software. Inhibition in the presence of drug was expressed as the fractional product in percent of control without drug. Inhibitory concentration 50% (IC50s), defined as the concentration of raltegravir that provokes a 50% inhibition, were calculated from inhibition curves fitted to experimental data with Prism 4.0 software (Graphpad software Inc, San Diego, USA).
RESULTS

Patients and viral load. Nine highly treatment-experienced patients, infected with multidrug-resistant virus, were treated with 400 mg bid raltegravir. Baseline characteristics of these nine patients were described in the Table 1. The median of plasma HIV-1 RNA was $5 \log_{10}$ copies/mL, ranged from 4.3 to 5.4. All of the nine patients harbored highly mutated viruses for NRTI, NNRTI and PI and their Genotypic Sensitivity Scores (GSS; number of ARV theoretically active) were $\leq 5$.

For six out of 9 patients (patients 1c, 2a-d and 3; Fig 1), the HIV-1 viral load decreased from baseline to become undetectable (VL<$1.60 \log_{10}$ copies/mL) in 4 to 16 weeks and then returned to baseline values except for the patient 2b. For the 3 remaining patients (1a-b and 4; Fig 1), the HIV-1 viral load showed only a slight decrease for the patient 4 (VL=$5.0 \log_{10}$ copies/mL) and it decreased but remained detectable for patients 1a and 1b ($2.86$ and $3.5 \log_{10}$ copies/mL, respectively).

HIV-1 integrase sequence analysis of clinical strains before raltegravir treatment. The complete nucleotide sequence of the 864 nt IN coding region was determined with clinical isolates obtained before the
initiation of anti-integrase treatment (D0) from the nine patients. The corresponding 288 amino acid (aa) sequences were compared to the HxB2 integrase reference sequence: divergence for the whole protein was calculated between 2.7 to 5.5%, depending of the patients (2.7% for patient 3, 3.8% for patients 1c, 2a, 2c and 4, 4.1% for patients 1a and 2b and 5.5% for patients 1b and 2d). The mutations G123S, R127K and N232D (with respect to HxB2) were present on D0 in all patients and D10E, E11D, A21T, A23V, D25E, V32I, S39C, V72I, L101I, I113V, S119P, T122I, A124T, T125A, I135V, V201I, T218S and L234I were present in at least 2 patients. Each patient also harbored specific aa sequence differences with respect to HxB2, including K7Q, M50L, I200L and I220L for patient 1a; L28I, S119G and I203M for patient 1b; S17N for patient 1c; K156N, V165I and I220V for patient 2a; M50I, T112I, A124N, L234V and S255R for patient 2b; K14E, V31I and I73V for patient 2c; V31M, K111R, G193E and S195G for patient 2d and R20K, I204V and T206S for patient 3 (Table 2).

Sequence analysis of clinical isolates during raltegravir treatment. For each patient, the integrase gene sequence was determined using isolates obtained at various time points during the follow-up. In all nine patients, one or two mutations appeared when the viral load increased back towards the initial viral load; however, four different patterns of mutations were
identified. Three patients (patients 1a, 1b and 1c) showed 2 mutations, G140S and Q148H that seemed to appear simultaneously, whereas four patients (patients 2a, 2b, 2c and 2d), showed only one mutation, N155H and patients 3 and 4 showed one mutation, E92Q and E157Q, respectively (Fig. 1 and Table 2).

Among the nine patients, four of them, each one showing a different profile (G140S + Q148H: patient 1a, N155H: patient 2a, E92Q: patient 3 and E157Q: patient 4), have been selected for an extended analysis of the integrase activity to estimate in vitro, the impact of the mutations on the 3’ processing and strand transfer activities.

Expression and sensitivity to raltegravir for the 3’ processing and the strand transfer activities of the wild-type integrases. The wild-type recombinant integrases from the four patients were produced and assayed in vitro for their sensitivity to raltegravir: all four enzymes were very sensitive to raltegravir (Fig. 2, panel B). IC₅₀s were determined by in vitro dose-response assays and were comparable (7-10nM) and in agreement with previously reported values for laboratory strains (31; M. Miller, M. Witmer, K. Stillmock, P. Felock, L. Ecto, J. Flynn, W. Schleif, G. Dornadula, R. Danovich and D. Hazuda, abstr. 16th Int. Conf. AIDS, Abstr. THAA0302, 2006).

Strand transfer activity was specifically inhibited whereas 3’ processing was
unaffected at inhibitor concentrations up to 100 times the IC50, thus confirming that raltegravir acts as a strand transfer inhibitor (15).

**Comparison of the sensitivities of wild-type and mutant integrases to raltegravir.** The mutant enzymes were first tested for their catalytic activities and compared to the efficiency of the wild-type enzyme (Table 3). All mutations diversely altered the functions of integrase *in vitro* (Fig. 2 and Table 3). The mutant E92Q was moderately impaired for both 3’ processing and strand transfer as it retained 76% and 90% of these activities respectively (Fig. 2A and Table 3). The mutant N155H expressed active 3’ processing (72% of wild-type) but was impaired for strand transfer (12% of wild-type). The mutant G140S + Q148H was strongly impaired for both activities, retaining only little residual activity (3% activity of wild type). The mutant E157Q was almost completely inactive for both 3’ processing and strand transfer. Mutant enzymes G140S + Q148H, N155H and E92Q retained sufficient activity to test their sensitivity to raltegravir (Fig. 2, panel B). However, the *in vitro* activity of mutant 4 was too low for the inhibitor to be tested.

All three mutant enzymes were more resistant to raltegravir than the parental enzymes: mutants G140S + Q148H and E92Q were 7 to 8 times more resistant and mutant N155H was more than 14 times more resistant (Fig. 2).
DISCUSSION

Raltegravir belongs to a new class of antiretroviral compound which targets HIV-1 integrase. It is currently undergoing late stage clinical trials in patients infected with multidrug-resistant HIV-1. We report integrase sequence diversity and evolution during viral escape in nine individuals infected with multidrug-resistant HIV-1.

Despite a high initial viral load, this one decreased rapidly to a minimum HIV-1 RNA titer, most often after 4 weeks of raltegravir treatment. For six patients, the treatment led to a substantial decrease of the viral load, whereas in three patients the decrease was weaker.

Initially, the integrase sequences showed little divergence from the reference HxB2 sequence, although there were numerous reverse transcriptase (RT) and protease mutations in these isolates from patients failing treatment. Indeed, it has been shown that there were some associations between RT mutations and IN mutations related to resistance to integrase inhibitors, in HAART-treated patients. For example, in the present study, the IN mutations at baseline included V165I in one patient and T206S in another which have previously been described to be associated with RT resistance mutations (F. Ceccherini-Silberstein, I. Malet, L. Fabeni, V. Svicher, C. Gori, S. Dimonte, S. Bono, A.
Artese, R. D’Arrigo, C. Katlama, A. Antinori, A. d’Arminio Monforte, V. Calvez, A. G. Marcelin, C. F. Perno, on behalf of the EuroGene HIV Network, Abstr. 5th European HIV Drug Resistance Workshop, abstr. 52, 2007). However, it is unclear whether this polymorphism can be implicated in the response profile to raltegravir treatment.

The kinetics of viral escape from the treatment were not exactly the same for the nine patients studied, but the viral load tends to return to initial values for most of them. However, it seems that there was no link between the profile of mutations and the kinetic of the viral load. When these nine patients escaped to the treatment, they harbored at least one mutation in the IN gene: E92Q, G140S+Q148H, N155H or E157Q.

IN reported mutations were associated with phenotypic resistance of the enzyme, thus confirming that integrase is the primary target of the inhibitor in vivo. The observation that the HIV-1 viral load was able to rebound to the pretherapeutic level for viruses harboring only a single mutation suggests that the genetic barrier to resistance of this compound is thin: the selection of only one mutation seems to be sufficient for virological failure of raltegravir treatment. Moreover, the absence of pharmacokinetic/pharmacodynamic relation for raltegravir does not argue for the interest of increasing the dose of raltegravir to overcome the resistance (29).
Selection of secondary mutations was not observed in this study; this contrasts with the findings of a sub-study of resistance conducted in a phase 2 trial, where secondary mutations seemed to appear after the selection of mutations in positions 148 or 155 (D. Hazuda, M.D. Miller, B. Y. Nguyen and J. Zhao, Abstr. XVI international HIV drug resistance workshop, abstr. 8, 2007).

In this same study, it was suggested that there were two genetic pathways for the development of resistance to raltegravir: one involving Q148H and the other N155H mutations. N155H is known to generate resistance to the IN inhibitor naphthyridine carboxamide (L-870812) (14) and G140S is involved in resistance to chicoric acid and the DKA family of IN inhibitors (23, 24). Some studies showed that the Q148 residue is implicated in interactions with the 5’ terminal of the viral DNA end allowing efficient integrase strand transfer (12, 20), suggesting that a mutation at this position could reduce IN activity. However, the present study suggests that, in addition to these both resistance profiles, there are other pathways associated with raltegravir resistance, involving E92Q or E157Q mutations.

The existence of several integrase resistance profiles is similar to what has been described for other ARV classes, such as NNRTIs. However, it is unknown what are the determinants of the evolution toward these different profiles. This could be related to different factors as the genetic polymorphism of the integrase gene, pharmacokinetic factors such as raltegravir trough levels or the sequence
of other genes encoding for proteins that being likely interact with the integrase as the reverse transcriptase (36, 37).

These findings argue strongly that raltegravir should only be used in combination with other active drugs. Most of the mutations we report severely impaired the function of the enzyme. It is unclear whether this lack of activity reflects an intrinsic property of the mutated enzyme or whether it is merely due to the purification procedure. Indeed, several factors, such as concentration, presence of cations and detergents, during purification appear to impact the ability of recombinant HIV-1 IN to perform efficient integration (35). The presence of a His-tag at the N-terminal extremity may also affect the activity of the enzymes since it is known that this sequence impacts the capability of integrases to form active oligomers (19). If it eventually turned out that, the virus was able to replicate despite encoding a catalytically inactive integrase and the mechanism involved would remain to be elucidated. Cellular factors such as LEDGF which stimulate integration in vitro could be involved (4). This type of phenomenon has already been described, for example, for protease inhibitors: viral mutations in gag cleavage sites are selected to overcome the decrease of viral fitness due the selection of resistance mutations in the HIV-1 protease.

At least four genetic profiles (E92Q, G140S + Q148H, N155H and E157Q) can be associated with in vivo treatment failure and resistance to raltegravir. These mutations led to enzymes strongly impaired in vitro in the absence of
raltegravir, strand transfer activity was affected and in some cases the 3’
processing was also impaired.
REFERENCES


FIG. 1. Kinetics of plasma HIV RNA copy number in 9 patients failing raltegravir therapy. The dotted line placed at 1.60 log_{10} copies/mL (40 copies/mL) shows the detection limit of the viral load assay (wt = wild-type, NA = non amplifiable).

FIG. 2. 3’ processing and strand transfer activities of recombinant integrases. (A) Activity of recombinant integrases. INs obtained from patient 3 on day 0 (IN_{wt}) and week 14 (IN_{E92Q}) were incubated for one hour at 37°C with 10nM of the 21-mer substrate in the presence of various concentrations of raltegravir which are shown above the lanes (IN=integrase, st.=strand, 3’ pro=3’ processing) (B) Comparison of inhibition curves obtained for isolates from patients 1, 2 and 3 (RI=Resistance Index). (●) data obtained for wild-type virus at D=0; (▲) data obtained for resistant viruses.
<table>
<thead>
<tr>
<th>Patient</th>
<th>HIV-1 Viral Loada (log10 copies/mL)</th>
<th>No. of CD4 (Cells/mm3)</th>
<th>Resistance mutations</th>
<th>GSSb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>NNRTI: G190S/G</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>NNRTI: V106I, Y188L</td>
<td>1</td>
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<td></td>
<td></td>
<td></td>
<td>NNRTI: K103N</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>NNRTI: V108I, Y181C, G190A</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>2</td>
</tr>
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<td></td>
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<td>NNRTI: -</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>NNRTI: Y181C, G190S</td>
<td>3</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>NNRTI: K101Q</td>
<td>5</td>
</tr>
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</table>

*a Levels of HIV RNA in plasma were determined by using the COBAS Ampliprep/COBAS TaqMan HIV-1 test.

b GSS: Genotypic Sensitivity Score, according to genotypic resistance testing interpreted with the French ANRS AC11 algorithm v16 (www.hivfrenchresistance.org).

Abbreviations: NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; ins = insertion.
TABLE 2. Evolution of IN amino-acids substitutions during the raltegravir therapy

<table>
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<tr>
<th>Patient</th>
<th>Subtype</th>
<th>Anti-HIV-1&lt;sup&gt;a&lt;/sup&gt; agents received at D0</th>
<th>No. of CD4&lt;sup&gt;b&lt;/sup&gt; (Cells/mm&lt;sup&gt;3&lt;/sup&gt;)</th>
<th>Viral Load&lt;sup&gt;b&lt;/sup&gt; (log&lt;sub&gt;10&lt;/sub&gt; copies/mL)</th>
<th>Time of therapy&lt;sup&gt;c&lt;/sup&gt;</th>
<th>N-term (2a)</th>
<th>Catalytic core domain</th>
<th>C-term (2a)</th>
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<td>1a</td>
<td>B</td>
<td>3TC, TDF, LPV/r, fos-APV/r</td>
<td>65</td>
<td>4.9</td>
<td>DO</td>
<td>Q E D</td>
<td>L K</td>
<td>L D</td>
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<td></td>
<td></td>
<td></td>
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<td>W4</td>
<td>E</td>
<td>T E</td>
<td>I</td>
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<td>1b</td>
<td>B</td>
<td>T20, TMC-125, DRV/r, 3TC</td>
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<td>5</td>
<td>D0</td>
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<td></td>
<td></td>
<td></td>
<td>13</td>
<td>3.5</td>
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<td>I</td>
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<td>E</td>
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<td>3TC, TDF, ABC, fos-APV/r</td>
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<td>2d</td>
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<td>3TC, T20, TPV/r</td>
<td>39</td>
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<td>E</td>
<td>I K V S A K</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>B</td>
<td>T20, ABC, 3TC, DRV/r</td>
<td>5</td>
<td>5.4</td>
<td>D0</td>
<td>E</td>
<td>K V S K</td>
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<td></td>
<td></td>
<td>175</td>
<td>1.6</td>
<td>W4</td>
<td>E</td>
<td>I K V S A K</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>B</td>
<td>TDF, FTC, LPV/r</td>
<td>1</td>
<td>5.4</td>
<td>D0</td>
<td>E</td>
<td>T V E I S T K</td>
<td>I</td>
</tr>
<tr>
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<td></td>
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<td>31</td>
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<td>W2</td>
<td>E</td>
<td>I K V S A K</td>
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<td>52</td>
<td>4.9</td>
<td>W4</td>
<td>E</td>
<td>I K V S A K</td>
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</tr>
</tbody>
</table>

<sup>a</sup> AZT, zidovudine; TDF, tenofovir; 3TC, lamivudine; ddi, didanosine; LPV/r, lopinavir; fos-APV, fos-amprenavir; ABC, abacavir; DRV/r, darunavir; FTC, emtriva; TPV/r, tipranavir; TMC-125, emtriva; IDV, indinavir.

<sup>b</sup> Levels of HIV RNA in plasma were determined by using the COBAS AmpliPrep/COBAS TaqMan HIV-1 test; the detection limit of the viral load assay is 1.60 log<sub>10</sub> copies/mL.

<sup>c</sup> Days 0 (D0), week 2 (W2), week 4 (W4), week 8 (W8), week 12 (W12), week 24 (W24).

ABC = non amplifiable.

<sup>d</sup> D = day, W = week. At D0, the integrase sequence was compared to the HXB2 reference sequence and the modified amino-acids are indicated. At the other times of the follow-up (W2 to W24), the dashes mean that there is no modification compared to the D0 sequence.
### TABLE 3. Activities of mutant integrases

<table>
<thead>
<tr>
<th>IN enzyme</th>
<th>3' processing&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Strand transfer&lt;sup&gt;a&lt;/sup&gt;</th>
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</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>100</td>
<td>100</td>
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<tr>
<td>Mutant</td>
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<tr>
<td>E92Q</td>
<td>76</td>
<td>90</td>
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<td>N155H</td>
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<td>12</td>
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<tr>
<td>G140S/Q148H</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

<sup>a</sup> 3' processing and strand transfer efficiency were expressed as a percentage of wild-type activity.