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Secondary mutations in viruses resistant to HIV-1 integrase inhibitors that restore viral infectivity and replication kinetics

Koichiro Nakahara[a], Chiaki Wakasa-Morimoto[a], Masanori Kobayashi[a], Shigeru Miki[a], Takeshi Noshi[a], Takahiro Seki[a], Mikiko Kanamori-Koyama[a], Shinobu Kawauchi[a], Akemi Suyama[a], Toshio Fujishita[b], Tomokazu Yoshinaga[a], Edward P. Garvey[c], Brian A. Johns[d], Scott A. Foster[c], Mark R. Underwood[c], Akihiko Sato[a,*], Tamio Fujiwara[a]

[a] Virology, Shionogi Research Laboratories, Shionogi & Co., Ltd., 2-5-1, Mishima, Settsu-shi, Osaka 566-0022, Japan
[b] Medicinal Chemistry, Shionogi Research Laboratories, Shionogi & Co., Ltd., Osaka 566-0022, Japan
[c] Virology, Infectious Disease Center of Excellence for Drug Discovery, GlaxoSmithKline, Research Triangle Park, NC 27709, USA
[d] Medicinal Chemistry, Infectious Disease Center of Excellence for Drug Discovery, GlaxoSmithKline, Research Triangle Park, NC 27709, USA

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ABSTRACT

Passage of HIV-1 in the presence of integrase inhibitors (INIs) generates resistant viruses that have mutations in the integrase region. Integrase-resistant mutations Q148K and Q148R were identified as primary mutations with the passage of HIV-1 IIIB in the presence of INIs S-1360 or S/GSK-364735, respectively. Secondary amino acid substitutions E138K or G140S were observed when passage with INI was continued. The role of these mutations was investigated with molecular clones. Relative to Q148K alone, Q148K/E138K had 2- and >6-fold increases in resistance to S-1360 and S/GSK-364735, respectively, and the double mutant had slightly better infectivity and replication kinetics. In contrast, Q148K/G140S and Q148R/E138K had nearly equivalent or slightly reduced fold resistance to the INI compared with their respective Q148 primary mutants, and had increases in infectivity and replication kinetics. Recovery of these surrogates of viral fitness coincided with the recovery of integration efficiency of viral DNA into the host cell chromosome for these double mutants. These data show that recovery of viral integration efficiency can be an important factor for the emergence and maintenance of INI-resistant mutations.

1. Introduction

Integrase (IN), one of three viral enzymes essential for HIV-1 replication, catalyzes integration of viral DNA into the host genome. The replication of HIV-1 can be suppressed by inhibiting this enzyme (Nakajima et al., 2001; Taddeo et al., 1994; Wiskerchen and Muesing, 1995). The antiviral activity of integrase inhibitors has been clinically demonstrated in HIV positive subjects (DeJesus et al., 2006; Markowitz et al., 2006), and INIs are expected to be included in highly active anti-retroviral therapy (HAART). However, even this new class of drug will encounter drug resistance, and it is important to know the identity of drug-resistant mutants and understand the evolution of mutations *in vitro* and in the clinic.

During the course of passage studies (Kobayashi et al., 2008), we isolated Q148K as a primary mutant resistant to S-1360 (Billich, 2003) which progressed with further passage to Q148K/E138K, and also isolated Q148R as a primary mutant resistant to S/GSK-364735 (Garvey et al., 2008) which progressed to Q148R/E138K and Q148R/G140S. These mutants were also isolated in our *in vitro* cell culture (Kobayashi et al., 2008) with L-870,810 and raltegravir, and have also been reported in clinical studies of raltegravir and elvitegravir (Hazuda et al., 2007; McColl et al., 2007). We report here the comparison of fold resistance of these mutant molecular clones, viral replication kinetics and relative viral infectivity. We also report the relative integration activity of these mutants measured by quantitative PCR assay.

2. Materials and methods

2.1. Compounds

S/GSK-364735, efavirenz and nelfinavir were synthesized at GlaxoSmithKline, Research Triangle Park, NC. S-1360 was synthesized at Shionogi Research Laboratories, Osaka, Japan. The structures of S/GSK-364735 and S-1360 are depicted in Fig. 1.

* Corresponding author. Tel.: +81 6 6382 2612; fax: +81 6 6382 2598.
E-mail address: akihiko.sato@shionogi.co.jp (A. Sato).

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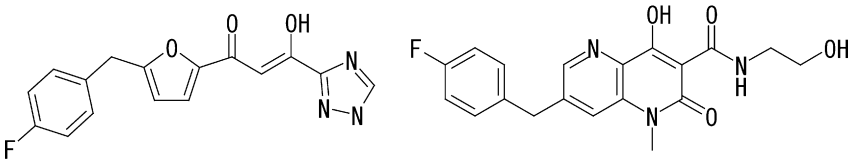

Fig. 1. Structure of S-1360 and S/GSK-364735.

2.2. Cells

The 293T cell line was obtained from ATCC. HeLa-CD4 cells carrying a reporter β-galactosidase gene driven by HIV-1 LTR were established by transfection of HeLa cells with CD4 and β-galactosidase expression vector (Isaka et al., 1999). Human T cell lines (Jurkat and MT-4) were obtained from Institute for Virus Research, Kyoto University. Peripheral blood mononuculear cells (PBMCs) derived from healthy donors were purchased from ALLCELLS.

2.3. Construction of integrase region recombinant HIV-1 molecular clones

The recombinant HIV-1 molecular clones were constructed as follows. The XbaI–EcoRI fragment from pNL-IN301 [pNL432 (Adachi et al., 1986) inserted XbaI site into 5′ end of IN region] was cloned into the XbaI–EcoRI site of cloning vector pUC18. *In vitro* mutagenesis was performed with the QuickChange site-directed mutagenesis kit (Stratagene) using pUC18 cloned with IN region as a template. The mutated XbaI–EcoRI fragment was amplified and ligated into pNL-IN301 to construct a recombinant HIV-1 molecular clone. The plasmids were subsequently transfected into 293T cells to generate infectious virus. Supernatants were harvested after 2 days of culture and stored as cell-free culture supernatants at −80 °C.

2.4. Reverse transcriptase (RT) assay

To quantitate HIV-1 infection (see below), RT activity was routinely assayed by using synthetic template-primers as described (Sato et al., 1995). Briefly, a 100 μL reaction mixture contained 50 mM Tris–HCl, pH 8.3, 150 mM KCl, 10 mM $MgCl_2$, 0.1% Nonidet P-40, 10 mM DDT (dithiothreitol), 5 μg/mL poly (rA), 1.25 μg/mL $(dT)_{12-18}$, 1 μCi [^{3}H]dTTP and 10 μL of each viral sample. After incubation at 37 °C for 3 h, reaction mixtures were chilled on ice and passed through a DEAE-Filtermat (Wallac) using a cell harvester. After washing with 0.25% phosphate buffer, radioactivities on the filter were determined by a MicroBeta TRILUX instrument (Amersham Pharmacia Biotech).

2.5. Susceptibility of the integrase mutant molecular clones against drugs

The susceptibility of the integrase mutant molecular clones to S-1360, S/GSK-364735 and efavirenz were assessed by HeLa-CD4 cells reporter assay. The test compounds were diluted to appropriate concentration with culture medium and HeLa-CD4 cell suspensions (2.5×10^4 cells/well) were dispensed into each 96-well plate. After incubation for 1 h, the viruses were added. The infection titer of virus was determined by the luminescent activity (RLU) of each virus and normalized the infection titer to about 1000–5000 RLU. After 3 days of incubation, the cells were lysed and supernatant of each well was used for measurement of RLU using the Reporter Assay Kit-βgal (TOYOBO). The RLU was measured using a MicroBeta TRILUX instrument. The concentration achieving 50% inhibition of HIV infection (EC_{50}) was calculated.

2.6. Viral replication kinetics in Jurkat cells

Jurkat cells (4×10^4) were infected with NL432 or INI-resistant viruses for 1 h at 37 °C, washed and cultured in 24-well plates (1 mL/well). Viral stocks were normalized by RT activity prior to infection (100,000 cpm/4×10^4 cells). The infected cells were subcultured as 10-fold dilutions twice a week, and virus production in culture supernatants was titrated for RT activity.

2.7. Viral replication kinetics in PBMCs

5×10^4 cells of PHA-stimulated PBMCs were exposed to each infectious clone for 1 h at 37 °C. Viral stocks were normalized by RT activity prior to infection (20,000 cpm/5×10^4 cells). Cells were washed with PBS and cultured in 200 μL of complete medium supplemented with PHA and IL-2 (Roche). Culture supernatants (10 μL) were removed at various times, followed by the addition of the equal volume of fresh complete medium. On day 7 of culture, 100 μL of culture medium was collected and the equal volume of fresh medium was replenished to ensure the optimal growth of the cells. Virus production in culture supernatants was titrated with RT activity.

2.8. Determination of relative infectivity of the integrase mutant molecular clones

Relative infectivity of the integrase mutant molecular clone was measured using HeLa-CD4 cells. WT or mutant viruses were diluted serially with culture medium, and HeLa-CD4 cell suspensions (2.5×10^4 cells/well) were dispensed into each plate. After 3 days of incubation, virus-induced β-galactosidase activity was measured as described above. To evaluate relative infectivity, virus-induced β-galactosidase activity was normalized by RT activity of input virus. The RT-normalized infectivity of wild type virus was defined as 100%, and relative infectivity of each resistant virus was calculated in proportion to its induced β-galactosidase activity.

2.9. Measurement of viral DNA species by quantitative PCR assay

293T cells were transfected with the NL432 or mutant plasmids to generate infectious viruses. Supernatant was filtered through 0.45-μm pore-size filters and treated with DNase I (Roche) at 20 U/ml for 60 min at room temperature to prevent plasmid DNA carryover. MT-4 cells were infected with HIV-1 NL432 or each mutant virus for 1 h and incubated with 1 μM nelfinavir to limit HIV replication to a single cycle until collection after 6 or 18 h of incubation. Samples collected after 6 h of incubation were prepared for total-DNA PCR to detect late RT products. Samples collected after 18 h of incubation were prepared for nested *Alu* PCR to detect

integrated provirus and for two-LTR PCR to detect two-LTR circles. Total cell DNA was extracted from infected cells with a DNeasy blood and tissue kit (Qiagen). The copy number of late RT products, 2LTR circles and integrated proviruses were determined using specific quantitative PCR methods reported by Brussel A and Butler SL, respectively, with minor modification (Brussel and Sonigo, 2003; Butler et al., 2001). The amount of late RT products was determined with primers that annealed in the U5 region of the LTR (MH531: TgTgTgCCCgTCTgTTgTgT) and in the 5′ end of the gag gene (MH532-NL: gAgTCCTgCgTCgAgAgATC) and specific probe (LRT-P: CAgTggCgCCCgAACAgggA). The 2LTR circles were amplified with primers that annealed in the R region of the 3′ LTR (MH535: AACTAgggAACCCACTgCTTAAg) and in the U3 region of the 5′ LTR (MH536: TCCACAgATCAAggATATCTTgTC) and LTR specific probe (MH603: ACACTACTTgAAgCACTCAAggCAAgCTTT). To quantify integrated HIV-1 DNA, a nested Alu PCR was performed. An LTR primer extended with a lambda phage-specific heel sequence at 5′ end of the oligonucleotide (L-M667: ATgCCACgTAAgCgAAACTCTggCTAACTAgggAACCCACTg) in the first amplification step was used. Alu-LTR sequences were amplified first with L-M667 and Alu sequence specific primer (SB704: TgCTgggATTACAggCgTgAg). The second round quantitative PCR was performed using the lambda-specific primer (Lambda T: ATgCCACgTAAgCgAAACT) and an LTR primer (AA55 M: gCTAgAgATTTTCCACACTgACTAA) and LTR specific probe (MH603: ACACTACTTgAAgCACTCAAggCAAgCTTT). Reactions were analyzed using the ABI Prism 7900HT-3 sequence detection system (Applied Biosystems).

2.10. Statistical analysis

The Student's *t* test was used for statistical analysis.

3. Results

3.1. Construction of integrase mutant molecular clones and their fold resistance to INIs

In order to investigate the significance of the secondary mutations isolated in passage experiments with S-1360 and S/GSK-364735 (Kobayashi et al., 2008), we constructed a series of single or double mutants and analyzed their fold resistance against these INIs (Table 1). Efavirenz was also analyzed as a control compound. Fold resistance of each INI-resistant virus against efavirenz was within two. In addition to the particular passage study previously reported, a similar separate study with S-1360 yielded the Q148K/G140S double mutant (Sato et al., unpublished data). The clones containing single substitutions with Q148K or Q148R showed a high degree of resistance against S-1360 and S/GSK-364735. On the other hand, mutant clones with E138K or G140S substitutions (which were not isolated *in vitro* or in the clinic) showed only slightly or no reduced sensitivity to S-1360 and S/GSK-364735.

Q148K/E138K and Q148K/G140S was identified in S-1360 passage study. The addition of E138K mutation to Q148K mutant virus caused a two-fold increase in resistance to S-1360, while the addition of G140S to Q148K slightly decreased resistance to this INI.

As for Q148R/E138K and Q148R/G140S isolated in S/GSK-364735 passage study, the addition of E138K to the Q148R mutant virus caused a two-fold increase in resistance to S/GSK-364735, while the addition of G140S caused a much higher (>6-fold) increase in resistance to S/GSK-364735.

Thus, the additional amino acid substitutions to the primary mutations Q148K/R did not necessarily confer further resistance against the INIs with which these mutant viruses were isolated.

3.2. Surrogates for viral fitness of integrase mutant molecular clones in the absence of INIs

Multi-cycle replication kinetics of these mutants were measured with Jurkat cells (Fig. 2a–d), in which HIV-1 grows more slowly than in MT-2 cells used for our isolation study (Kobayashi et al., 2008), and suitable to distinguish the difference of viral replication among the mutants. The double mutant viruses Q148K/E138K (Fig. 2a), Q148K/G140S (Fig. 2b) and Q148R/E138K (Fig. 2c) showed higher replication than their primary single mutants (ex, Q148K or Q148R). Replication of Q148K, Q148R and Q148R/G140S (Fig. 2d) was not observed in Jurkat cells during the period of time examined.

Multi-cycle replication kinetics of mutant viruses in human PBMC were also measured (Fig. 3a–d) to compare with those in Jurkat cells and for possibly greater clinical relevance. In PBMC, Q148R replicated better than Q148K. The double mutant virus Q148K/G140S (Fig. 3b) showed a pronounced recovery of replication compared with Q148K. In addition, Q148R/E138K (Fig. 3c) displayed an improvement in replication at early time points compared with Q148R, and Q148K/E138K (Fig. 3a) was only slightly improved in the replication compared with Q148K. The replication of Q148R/G140S was impaired compared vs. Q148R (Fig. 3d). Thus, these overall replication kinetics patterns in PBMCs were similar to patterns in Jurkat cells.

Relative infectivity of these mutant viruses was also measured using the single cycle reporter assay with HeLa-CD4 cells. With this assay, the infectivity of integrase active site D64N/D116N mutant virus was about 2% of wild type (data not shown). Fig. 4 shows the relative infectivity of integrase mutants in the absence of INIs. The infectivity of Q148K mutant virus was reduced to 11% of wild-type virus and the addition of E138K or G140S mutation partially restored its infectivity to 36% or 41% of wild-type virus, respectively. The infectivity of Q148R mutant virus was reduced to 19 % of wild-

Table 1
Generation of integrase mutant molecular clones and their sensitivity to S-1360, S/GSK-364735 and efavirenz.

HIV-1 clone substitution	EC_{50}[a], nM (fold resistance[b])		
	S-1360	S/GSK-364735	Efavirenz
WT	330	3.6	1.7
Q148K	20790 (63)	818 (225)	3.6 (2.1)
Q148K/E138K	42900 (130)	>4514 (>1254)	2.0 (1.2)
Q148K/G140S	12210 (37)	333 (91)	2.2 (1.3)
Q148R	27720 (84)	273 (75)	3.2 (1.9)
Q148R/E138K	7590 (23)	539 (148)	1.7 (1.0)
Q148R/G140S	12210 (37)	2023 (562)	2.6 (1.5)
E138K	462 (1.4)	3.9 (1.1)	1.1 (0.67)
G140S	790 (2.4)	18 (4.9)	1.9 (1.1)

[a] Antiviral activity was determined using HeLa-CD4 cell reporter assay. Data shown were means from at least three independent experiments.
[b] Fold increase in EC_{50} compared to that of WT-NL432.

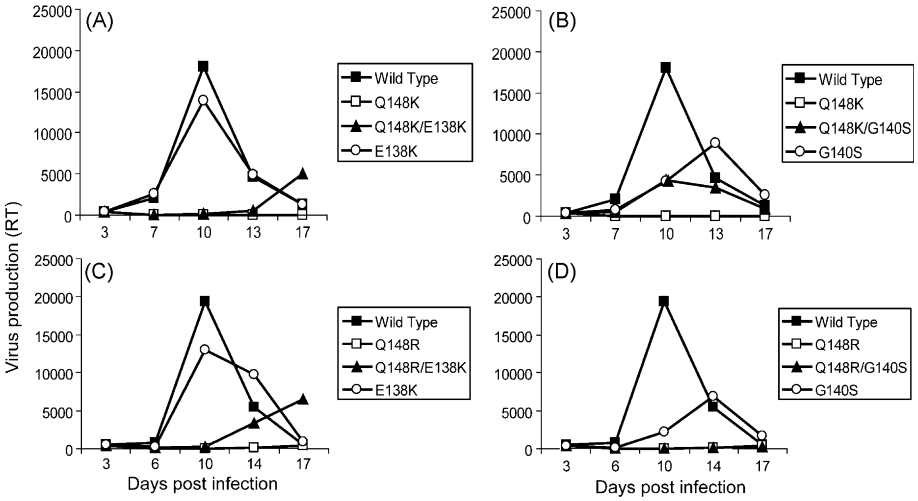

Fig. 2. Replication kinetics in Jurkat cells of mutant viruses resistant to INIs. The supernatant of each mutant was assayed for RT activity at the indicated time points. Three independent experiments generated the same results. Representative data are shown comparing the replication kinetics of double mutants (vs. respective single mutants) for: (a) Q148K/E138K, (b) Q148K/G140S, (c) Q148R/E138K, and (d) Q148R/G140S.

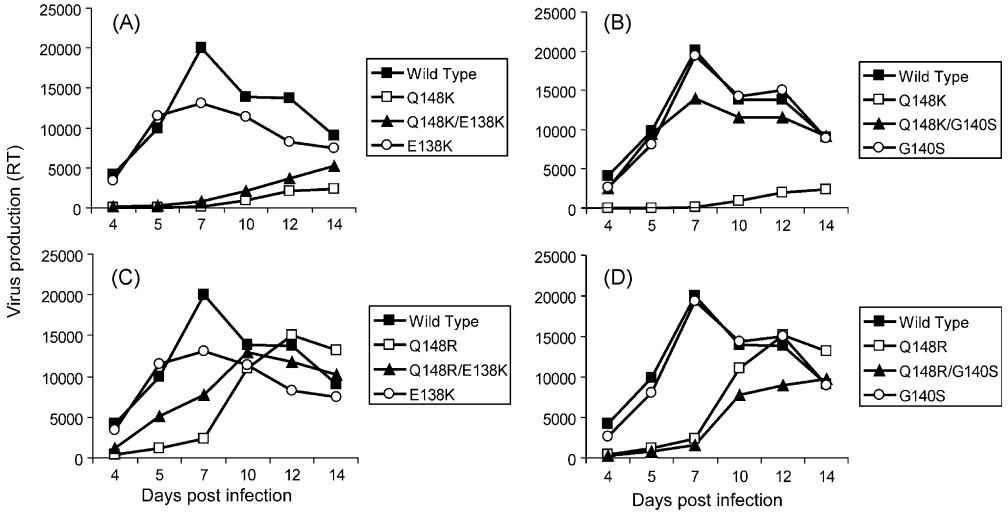

Fig. 3. Replication kinetics in PBMC of mutant viruses resistant to INIs. The supernatant of each mutant was assayed for RT activity at the indicated time points. Two independent experiments generated the same results. Representative data are shown comparing the replication kinetics of double mutants (vs. respective single mutants) for: (a) Q148K/E138K, (b) Q148K/G140S, (c) Q148R/E138K, and (d) Q148R/G140S.

type virus and the addition of E138K mutation partially restored its infectivity to 40% of wild type virus. Partial recovery in infectivity by these secondary mutations was not observed in Q148R/G140S. By statistical analysis, *p*-values of Q148K vs. Q148K/E138K, Q148K vs. Q148K/G140 and Q148R vs. Q148R/E138K were 0.001, 0.003 and <0.001, respectively, indicating these differences were statistically significant. In constrast, relative infectivity of Q148R and Q148R/G140S were not statistically different (*p*-value of 0.898). These infectivity data correlated well with the replication kinetics data insofar as the three double mutants all showed improvement in infectivity vs. to the lack of improvement in the Q148R/G140S mutant.

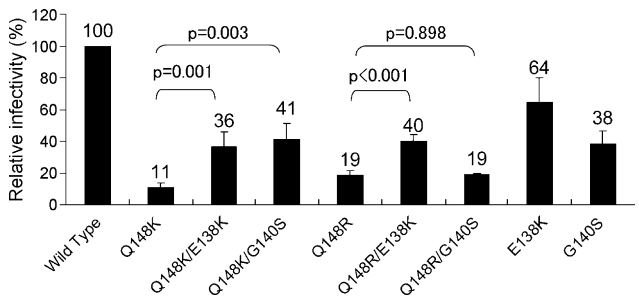

Fig. 4. Relative infectivity in HeLa-CD4 assay of mutant viruses resistant to INIs. Each bar represents mean value of five independent experiments. Error bars represent standard deviation. Statistical analysis was shown as *p*-value.

3.3. Viral DNA integration activity of integrase mutant molecular clones

In order to examine which step of the HIV lifecycle was impaired or improved with these integrase mutants, three viral DNA species, late RT products (total DNA), integrated DNA, and 2-LTR circular DNA from MT-4 cells infected with mutant viruses were assayed by quantitative PCR. As shown in Fig. 5a, the relative amount of total viral DNA from all of the tested integrase mutant viruses was similar and close to that of wild type. The integration efficiency of viral DNA of Q148K/E138K, Q148K/G140S and Q148R/E138K was greater than that of the corresponding single

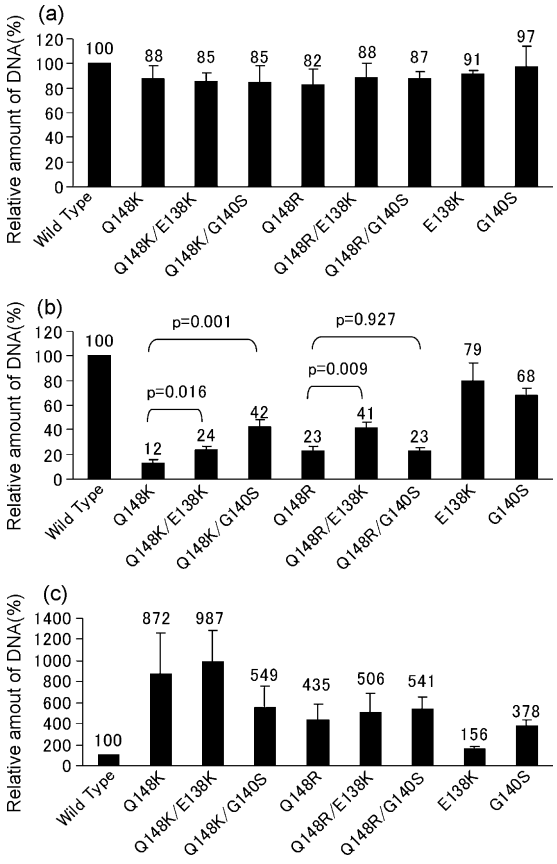

Fig. 5. Effects of integrase mutations on the amounts of various forms of viral DNA in the HIV infected MT-4 cells. MT-4 cells were infected with HIV-1 wild type NL432 or integrase mutants to determine the effects of mutations on: (a) total viral DNA, (b) integrated viral DNA, and (c) 2-LTR circular viral DNA. Y axis of each graph represents relative amount (%) of DNA vs. control. Each bar represents mean value of three independent experiments. Error bars represent standard deviation. Statistical analysis was shown as *p*-value.

mutant Q148K and Q148R, respectively (Fig. 5b). *p*-Values of Q148K vs. Q148K/E138K, Q148K vs. Q148K/G140S, Q148R vs. Q148R/E138K and Q148R vs. Q148R/G140S were 0.016, 0.001, 0.009 and 0.927, respectively. *p*-Values in the quantitative assay of integrated viral DNA correlated well with the results of relative infectivity measured in the HeLa-CD4 assay (Fig. 4). The amount of 2-LTR circles and integrated DNA of these mutants were inversely correlated (Fig. 5c). This result is consistent with previous reports that cells infected with integrase active-site mutants contained a higher level of viral 2-LTR circles compared to WT-infected cells (Ansari-Lari et al., 1995; Engelman et al., 1995; Wiskerchen and Muesing, 1995).

4. Discussion

Our study had three main findings; (i) viral fitness of HIV-1 measured by relative infectivity and replication kinetics surrogates was reduced by introduction of the primary resistance mutations Q148K or Q148R, (ii) the reduced infectivity and impaired replication kinetics of these singly mutated viruses partially recovered by addition of secondary mutations, E138K or G140S to Q148K and E138K to Q148R, and (iii) viral fitness measured by surrogates of these integrase mutant viruses correlated with the amount of integrated viral DNA. The exception was Q148R/G140S which showed similar fitness as Q148R. This lack of an effect on fitness due to the secondary mutation at G140S was counterbalanced by a 7-fold increase in resistance to S/GSK-364735.

Under the selective pressure of inhibitor, single mutations that yield high fold resistance, such as Q148K or Q148R, emerged first. G140S or E138K, which had little or no impact on fold resistance, did not emerge as primary mutations. The fold resistances of certain double mutants (Q148K/G140S and Q148R/E138K) were either similar to or significantly less than that of the original single mutants. Thus, the reason for the emergence of these double mutants was not simply a further increase in fold resistance; the secondary mutations also provided a partial recovery in both replication kinetics and viral infectivity. The partial restoration of infectivity and replication paralleled the partial recovery of the integration efficiency, measured by the amount of integrated viral DNA. Thus, the data are consistent with these primary mutations in integrase causing a reduced integration activity and secondary mutations partially restoring the impaired integration activity, which in turn restored the replication kinetics and the viral infectivity.

Taken together, explanations for the emergence of these secondary mutations can be hypothesized as follows; Q148K/G140S evolved from Q148K due to improvements of replication/infectivity, Q148R/E138K evolved from Q148R also due to the recovery of replication/infectivity and a minor contribution of increase in resistance (2-fold to S/GSK-364735), Q148K/E138K evolved from Q148K due to both recovery of replication/infectivity (which detected at longer periods of culture) and a minor increase in resistance (2-fold to S-1360), and Q148R/G140S evolved from Q148R solely due to the increase in resistance to S/GSK364735 (7-fold).

In spite of the relatively good replication of Q148K/G140S, the frequency of emergence of this mutant was very low. Interestingly, the fold resistance of this double mutant against both S-1360 and S/GSK-364735 was lower than its original primary mutant Q148K. These data suggest that the decrease of resistance against an inhibitor could negatively affect the addition of a second mutation.

Partial restoration of the decreased replication capacity of primary mutations by secondary mutations has already been reported in viruses resistant to other classes of anti-HIV drugs (Dykes and Demeter, 2007). Our report is another example with INIs that suggests secondary mutations within integrase can at least partially compensate for the deleterious effects of primary mutations.

Although it is presently unknown how any of these mutations alter integrase at the molecular level, it has been hypothesized that the flexibility of integrase's surface loop structure of 140–149 is necessary for activity (Greenwald et al., 1999) and that mutations within this loop may affect its activity (Lu et al., 2005). Mutant integrase proteins G149A or G140A/G149A have been shown to lose their IN activity although they retained near wild-type DNA binding activity (Greenwald et al., 1999). Therefore, it is reasonable to consider that other single point mutations in this loop may have similar deleterious effects. Furthermore, secondary amino acid substitutions of G140 or E138 might restore some of the activity lost by a primary mutation by further affecting the flexible loop structure. Interestingly, Lu et al. (2005) reported that a secondary E138K substitution partially restored the negative effect of Q62A and Q62K mutations on replication kinetics.

Several reports have described either direct interaction between IN and RT or effects of IN on viral DNA synthesis (Padow et al., 2003; Tasara et al., 2001; Wu et al., 1999). Such results formally raise the possibility that IN mutations may have pleiotropic effects beyond IN catalytic activity and specifically on RT, which may explain a decrease of infectivity. However, the amount of late RT products were not affected in any mutants isolated in our study, and the recovery of viral replication kinetics and relative infectivity of mutant viruses was clearly dependent on the recovery of IN activity of these mutants.

Because S-1360 and S/GSK-364735 did not progress far enough into clinical development to isolate resistant mutations in patients treated with these inhibitors, we cannot compare their *in vitro* viral resistance with any clinical data. However, the mutations analyzed in this paper were similar or the same as those identified in other INI clinical trials (Hazuda et al., 2007; McColl et al., 2007). For example, Q148K/G140S mutation was identified in elvitegravir-treated patients in clinical trials (McColl et al., 2007), and Q148K/E138K and Q148R/E138K were reported in raltegravir clinical studies (Hazuda et al., 2007). For current and future integrase inhibitors, it is important to examine the profiles of drug-resistant mutants *in vitro* both in terms of fold resistance and replication capacity, with the goal of eventually understanding the correlation between *in vitro* and clinical resistance in this important new class of HIV therapy.

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References

Adachi, A., Gendelman, H.E., Koenig, S., Folks, T., Willey, R., Rabson, A., Martin, M.A., 1986. Production of acquired immunodeficiency syndrome-associated retrovirus in human and nonhuman cells transfected with an infectious molecular clone. J. Virol. 59, 284–291.

Ansari-Lari, M.A., Donehower, L.A., Gibbs, R.A., 1995. Analysis of human immunodeficiency virus type 1 integrase mutants. Virology 213, 680.

Billich, A., 2003. S-1360 Shionogi-GlaxoSmithKline. Curr. Opin. Investig. Drugs 4, 206–219.

Brussel, A., Sonigo, P., 2003. Analysis of early human immunodeficiency virus type 1 DNA synthesis by use of a new sensitive assay for quantifying integrated provirus. J. Virol. 77, 10119–10124.

Butler, S.L., Hansen, M.S., Bushman, F.D., 2001. A quantitative assay for HIV DNA integration in vivo. Nat. Med. 7, 631–634.

DeJesus, E., Berger, D., Markowitz, M., Cohen, C., Hawkins, T., Ruane, P., Elion, R., Farthing, C., Zhong, L., Cheng, A.K., McColl, D., Kearney, B.P., 2006. Antiviral activity, pharmacokinetics, and dose response of the HIV-1 integrase inhibitor GS-9137 (JTK-303) in treatment-naive and treatment-experienced patients. J. Acquir. Immune Defic. Syndr. 43, 1–5.

Dykes, C., Demeter, L.M., 2007. Clinical significance of human immunodeficiency virus type 1 replication fitness. Clin. Microbiol. Rev. 20, 550–578.

Engelman, A., Englund, G., Orenstein, J.M., Martin, M.A., Craigie, R., 1995. Multiple effects of mutations in human immunodeficiency virus type 1 integrase on viral replication. J. Virol. 69, 2729–2736.

Garvey, E.P., Johns, B.A., Gartland, M.J., Foster, S.A., Miller, W.H., Ferris, R.G., Hazen, R.J., Underwood, M.R., Boros, E.E., Thompson, J.B., Weatherhead, J.G., Koble, C.S., Allen, S.H., Schaller, L.T., Sherrill, R.G., Yoshinaga, T., Kobayashi, M., Wakasa-Morimoto, C., Miki, S., Nakahara, K., Noshi, T., Sato, A., Fujiwara, T., 2008. The naphthyridinone GSK364735 is a novel, potent human immunodeficiency virus type 1 integrase inhibitor and antiretroviral. Antimicrob. Agents Chemother. 52, 901–908.

Greenwald, J., Le, V., Butler, S.L., Bushman, F.D., Choe, S., 1999. The mobility of an HIV-1 integrase active site loop is correlated with catalytic activity. Biochemistry 38, 8892–8898.

Hazuda, D.J., Miller, M.D., Nguyen, B.Y., Zhao, J., 2007. Resistance to the HIV-integrase inhibitor raltegravir: analysis of protocol 005, a phase 2 study in patients with triple-class resistant HIV-1 infection. XVI International Drug Resistance Workshop. Antiviral Ther. 12, S10.

Isaka, Y., Sato, A., Miki, S., Kawauchi, S., Sakaida, H., Hori, T., Uchiyama, T., Adachi, A., Hayami, M., Fujiwara, T., Yoshie, O., 1999. Small amino acid changes in the V3 loop of human immunodeficiency virus type 2 determines the coreceptor usage for CXCR4 and CCR5. Virology 264, 237–243.

Kobayashi, M., Nakahara, K., Seki, T., Miki, S., Kawauchi, S., Suyama, A., Wakasa-Morimoto, C., Kodama, M., Endoh, T., Oosugi, E., Matsushita, Y., Murai, H., Fujishita, T., Yoshinaga, T., Garvey, E., Foster, S., Underwood, M., Johns, B., Sato, A., Fujiwara, T., 2008. Selection of diverse and clinically relevant integrase inhibitor-resistant human immunodeficiency virus type 1 mutants. Antivir. Res. 80, 213–222.

Lu, R., Limon, A., Ghory, H.Z., Engelman, A., 2005. Genetic analyses of DNA-binding mutants in the catalytic core domain of human immunodeficiency virus type 1 integrase. J. Virol. 79, 2493–2505.

Markowitz, M., Morales-Ramirez, J.O., Nguyen, B.Y., Kovacs, C.M., Steigbigel, R.T., Cooper, D.A., Liporace, R., Schwartz, R., Isaacs, R., Gilde, L.R., Wenning, L., Zhao, J., Teppler, H., 2006. Antiretroviral activity, pharmacokinetics, and tolerability of MK-0518, a novel inhibitor of HIV-1 integrase, dosed as monotherapy for 10 days in treatment-naive HIV-1-infected individuals. J. Acquir. Immune Defic. Syndr. 43, 509–515.

McColl, D.J., Fransen, S., Gupta, S., Parkin, N., Margot, N., Ledford, R., Chen, J., Chuck, S., Cheng, A.K., Miller, M.D., 2007. Resistance and cross-resistance to first generation integrase inhibitors: insights from a phase 2 study of elvitegravir (GS-9137). XVI International Drug Resistance Workshop. Antiviral Ther. 12, S11.

Nakajima, N., Lu, R., Engelman, A., 2001. Human immunodeficiency virus type 1 replication in the absence of integrase-mediated dna recombination: definition of permissive and nonpermissive T-cell lines. J. Virol. 75, 7944–7955.

Padow, M., Lai, L., Deivanayagam, C., DeLucas, L.J., Weiss, R.B., Dunn, D.M., Wu, X., Kappes, J.C., 2003. Replication of chimeric human immunodeficiency virus type 1 (HIV-1) containing HIV-2 integrase (IN): naturally selected mutations in IN augment DNA synthesis. J. Virol. 77, 11050–11059.

Sato, A., Kodama, M., Abe, K., Miki, S., Nishimura, M., Suyama, A., Ogata, M., Toyoda, T., Sugimoto, H., Yoshie, O., Fujiwara, T., 1995. A simple and rapid method for preliminary evaluation of in vivo efficacy of anti-HIV compounds in mice. Antivir. Res. 27, 151–163.

Taddeo, B., Haseltine, W.A., Farnet, C.M., 1994. Integrase mutants of human immunodeficiency virus type 1 with a specific defect in integration. J. Virol. 68, 8401–8405.

Tasara, T., Maga, G., Hottiger, M.O., Hubscher, U., 2001. HIV-1 reverse transcriptase and integrase enzymes physically interact and inhibit each other. FEBS Lett. 507, 39–44.

Wiskerchen, M., Muesing, M.A., 1995. Human immunodeficiency virus type 1 integrase: effects of mutations on viral ability to integrate, direct viral gene expression from unintegrated viral DNA templates, and sustain viral propagation in primary cells. J. Virol. 69, 376–386.

Wu, X., Liu, H., Xiao, H., Conway, J.A., Hehl, E., Kalpana, G.V., Prasad, V., Kappes, J.C., 1999. Human immunodeficiency virus type 1 integrase protein promotes reverse transcription through specific interactions with the nucleoprotein reverse transcription complex. J. Virol. 73, 2126–2135.