Dolutegravir (S/GSK1349572) Exhibits Significantly Slower Dissociation than Raltegravir and Elvitegravir from Wild-Type and Integrase Inhibitor-Resistant HIV-1 Integrase-DNA Complexes

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The integrase inhibitor (INI) dolutegravir (DTG; S/GSK1349572) has significant activity against HIV-1 isolates with raltegravir (RAL)- and elvitegravir (ELV)-associated resistance mutations. As an initial step in characterizing the different resistance profiles of DTG, RAL, and ELV, we determined the dissociation rates of these INIs with integrase (IN)-DNA complexes containing a broad panel of IN proteins, including IN substitutions corresponding to signature RAL and ELV resistance mutations. DTG dissociates slowly from a wild-type IN-DNA complex at 37°C with an off-rate of 2.7 × 10⁻⁶ s⁻¹ and a dissociative half-life (t₁/₂) of 71 h, significantly longer than the half-lives for RAL (8.8 h) and ELV (2.7 h). Prolonged binding (t₁/₂) at least 5 h was observed for DTG with IN-DNA complexes containing E92, Y143, Q148, and N155 substitutions. The addition of a second substitution to either Q148 or N155 typically resulted in an increase in the off-rate compared to that with the single substitution. For all of the IN substitutions tested, the off-rate of DTG from IN-DNA complexes was significantly slower (from 5 to 40 times slower) than the off-rate of RAL or ELV. These data are consistent with the potential for DTG to have a higher genetic barrier to resistance, provide evidence that the INI off-rate may be an important component of the mechanism of INI resistance, and suggest that the slow dissociation of DTG may contribute to its distinctive resistance profile.

Improvements in antiretroviral therapy have resulted in major advances in longevity and quality of life for HIV-infected patients. However, there is still a need to augment the anti-HIV armament: for example, by increasing tolerability and ease of dosing, maximizing potency, providing forgiveness in the face of adherence difficulties, and improving resistance profiles. The latest addition to anti-HIV therapy is inhibition of end processing, which removes the final 2 nucleotides (e.g., 5'-GT) from each end of the viral cDNA, and insertion of the two viral 3' DNA ends into opposite strands of the host DNA (strand transfer). Several compounds that specifically block strand transfer of the viral cDNA into the host DNA have been shown to be efficacious in vivo (reviewed in references 3 and 36). Raltegravir (RAL), which was approved by the FDA in 2007, was the first marketed IN inhibitor (INI), and elvitegravir (ELV) and dolutegravir (DTG; S/GSK1349572) are in late stages of development (Fig. 1). All three molecules have a common two-metal-binding motif by which the two essential metals at the IN catalytic site are bound by the inhibitor.

Resistance is typically the primary chink in an antiretroviral agent’s armor. Resistance mutations have been identified for both RAL and ELV during in vitro passaging experiments (20, 21, 30, 40, 43, 47) and in clinical studies (5, 35, 36, 45). Primary resistance mutations observed within the IN open reading frame include substitutions at T66 (ELV), E92 (ELV), Y143 (RAL), Q148 (both drugs), and N155 (both drugs) (reviewed in references 3 and 36). Multiple secondary mutations which may increase resistance and/or compensate for fitness defects caused by the primary mutations have also been identified. Overall, both RAL and ELV largely share resistance profiles (3, 30, 36, 37), and it was demonstrated that subjects experiencing virologic failure during therapy with ELV did not respond to treatment with RAL (10). Understanding resistance mechanisms may practically assist in optimizing the design and development of new drugs with improved resistance profiles, help define how to best use anti-HIV agents in the clinic, and inform on the potential that HIV might require multiple mutations to achieve resistance for a drug which may in turn be predictive of a higher barrier to resistance in vivo.

Characterizing the mechanisms of HIV drug resistance can involve many methods, including passage of virus in cell culture in the presence of a drug to select resistance, examination of resistance profiles observed in virus isolates from subjects experiencing virologic failure in the clinic, evaluation of the relative contributions of mutations to resistance and to resto-

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nucleotides 5'-biotinylated, 3'-processed plus-strand DNA and 50 μM minus-strand DNA at 95°C for 5 min in 50 mM 3-(N-morpholino)propanesulfonic acid (MOPS (pH 7.2)), 50 mM NaCl, and 10 mM MgCl₂, followed by cooling at room temperature for several hours. The DNA duplexes were attached via the 5'-biotin linker on the plus strand to streptavidin-coated SPA imaging beads by incubating 2 μM biotinylated DNA and 20 mg/ml beads in 25 mM MOPS (pH 7.2) on a Nutorax mixer for 80 min at room temperature. Unbound DNA was removed by two rounds of centrifugation and resuspension of the DNA-bead complex in 50 mM MOPS (pH 7.2), 50 mM NaCl, and 10 mM MgCl₂. The final bead concentration was 40 mg/ml, and the DNA-bead complex was stored at 4°C until needed.

IN-DNA-bead complexes were formed by incubating 1.0 to 2.8 μM wild-type or mutant IN protein with 5.2 mg/ml DNA-bead complex in 25 mM MOPS (pH 7.2), 23 mM NaCl, 10 mM MgCl₂, 10% dimethyl sulfoxide (DMSO), and 10 mM dithiothreitol for 8 min at 37°C. Unbound protein was removed by two rounds of centrifugation and resuspension in 25 mM MOPS (pH 7.2), 23 mM NaCl, and 10 mM MgCl₂, with the final bead concentration being 1.5 mg/ml. INI binding was initiated by mixing 140 μl of 1.5 mg/ml IN-DNA-bead complex (assay wells) or 140 μl of 1.5 mg/ml DNA-bead complex (control wells) and 10 μl of 600 nM [3H]-labeled DTG, RAL, or ELV in a 96-well microtiter plate. The plate was sealed and incubated overnight at room temperature to obtain maximum binding. The plate was placed in a 37°C incubator for 1 h prior to the addition of unlabeled INI. For the dissociation and dissociation control wells, 10 μl of 640 μM unlabeled INI in 25 mM MOPS (pH 7.2), 23 mM NaCl, 10 mM MgCl₂, and 8% DMSO was added to give a final concentration of 40 μM unlabeled DTG, RAL, or ELV. To maintain a consistent DMSO concentration, 10 μl of 25 mM MOPS (pH 7.2), 25 mM NaCl, 10 mM MgCl₂, and 8% DMSO was added to the high-signal and high-signal-control wells. Dissociation of [3H]-labeled INIs at 37°C was monitored for up to 3 weeks using a ViewLux charge-coupled-device imager (PerkinElmer), with the plate being maintained in a 37°C incubator between time points. The signals for duplicate wells were averaged, and the signal from control wells, which represents nonspecific binding of the [3H]-labeled INI to the DNA-bead complex, was subtracted from the high signal (the high-signal control was DNA-bead, 40 nM [3H]-labeled INI, and 0.5% DMSO) and the dissociation wells (the dissociation control was DNA-bead, 40 nM [3H]-labeled INI, 40 μM INI, and 0.5% DMSO). Relative binding was calculated as the ratio of the background-subtracted dissociation signal to background-subtracted high signal and was fit with the equation $RB = EP + \Delta RB \cdot e^{-kt}$, using the SigmoidPlat (version 8.0) program (Systat Software, Chicago, IL), where $RB$ is the relative binding at time $t$, $EP$ is the relative binding endpoint, $\Delta RB$ is the change in relative binding, and $k$ is the rate constant for dissociation. The endpoint was allowed to float, except for cases of extremely slow dissociation, when it was fixed at 0.1. Dissociative half-life $t_{1/2}$ values were calculated from $ln2/k$, using the mean $k$ value from multiple experiments. Statistical analysis was performed with the JMP (version 9) program (SAS Institute, Cary, NC). $P$ values were calculated from the log$_{10}(k)$ using all of the individual off-rate determinations and the least-squares means differences Student’s $t$ test ($n = 0.05, t = 1.9835$).

**Strand transfer assay.** To determine the catalytic stability of the IN-DNA complex, IN-DNA-bead complexes were formed as described above for wild-type, E90Q, Y143R, Q148H, N155H, E90Q/N155H, E138K/Q148R, and G140S/Q148H INs. After resuspension of the beads at 1.5 mg/ml, the IN-DNA-bead solution was put into a 37°C incubator and strand transfer activity was determined at various time points. The strand transfer assay was conducted as described previously (2), except that DMSO was omitted and the reaction was quenched after 30 min with 25 mM MOPS (pH 7), 50 mM EDTA, and 500 mM NaCl.

**RESULTS**

INI dissociation from wild-type IN-DNA complex. To determine rate of INI dissociation from IN-DNA complexes, [3H]DTG, [3H]RAL, and [3H]ELV were used in a SPA format (22, 23, 32). All experiments were performed at 37°C to approximate physiological conditions. Binding of the [3H]-labeled INI to the IN-DNA-bead complex resulted in a high signal, as shown in Fig. 2 for [3H]DTG and a wild-type IN-
DNA complex. There was no decrease in the high signal over time, indicating that the [3H]DTG-IN-DNA-bead complex was stable for more than 2 weeks at 37°C with wild-type IN. Dissociation of the [3H]labeled INI was monitored by adding excess unlabeled INI to block rebinding of the released [3H]-labeled INI, which resulted in a decrease in signal (Fig. 2 for DTG). Relative binding, the proportion of [3H]-labeled INI remaining bound to the IN-DNA-bead complex, was calculated for each time point (see Fig. 3A for wild-type IN). This calculation accounted for changes in the signal due to mixing and settling of the bead complexes and for changes in the signal from nonspecific binding of the [3H]-labeled INI to the imaging beads. Separate controls were used for the nonspecific background in the presence and absence of unlabeled INI due to a time-dependent decrease in the signal in the presence of unlabeled INI.

Dissociation of DTG from the wild-type IN-DNA complex was slow, with a \( k_{\text{off}} \) of \((2.7 \pm 0.4) \times 10^{-6} \text{ s}^{-1}\) (Table 1). Both RAL and ELV dissociated more quickly (\( P < 0.0001 \)) than DTG (Fig. 3A), with \( k_{\text{off}} \) values of \((22 \pm 2) \times 10^{-6} \text{ s}^{-1}\) for RAL and \((71 \pm 4) \times 10^{-6} \text{ s}^{-1}\) for ELV (Table 1). The dissociative \( t_{1/2} \) calculated for DTG was 71 h, which was 8 times longer than that of RAL (\( t_{1/2} \), 8.8 h) and 26 times longer than that of ELV (\( t_{1/2} \), 2.7 h) (Table 2). The dissociative half-life value that we measured for RAL with the wild-type IN-DNA complex at 37°C was similar to previously reported values of 7.3 h obtained at 37°C (23) and 11.0 h obtained at 25°C (28). A dissociative half-life value of 11.1 h was previously reported for ELV at 25°C (28).

**Effect of single IN substitutions on INI dissociation from IN-DNA complexes.** Dissociation experiments were also performed with IN-DNA complexes containing different IN substitutions to determine if changes at specific residues impact INI dissociation. Most of the IN substitutions studied here significantly (\( P < 0.0001 \)) increased the \( k_{\text{off}} \) of the INIs from the IN-DNA complexes compared to wild type (Table 1; Fig. 3).
4). Despite the effect of single IN substitutions on DTG dissociation from IN-DNA complexes, prolonged binding with $t_{1/2}$ values of 5.2 to 8.4 h (Table 2) was observed for DTG with all of the single-residue substitutions that were tested. Of the key INI resistance mutations, the N155H and Q148H/K/R substitutions had the greatest effect on DTG dissociation from IN-DNA complexes, having $k_{off}$ values of $18 \times 10^{-6}$ to $37 \times 10^{-6}$ s$^{-1}$, representing a 6.7- to 13.7-fold increase in $k_{off}$ relative to the wild-type IN-DNA complex (Table 1). The dissociation of RAL and ELV from the N155H and Q148H/K/R IN-DNA complexes was significantly ($P < 0.0001$) faster than that of DTG (Fig. 3C and D and 4), except for Q148H and G140S/Q148H with RAL from IN-DNA complexes containing double IN substitutions (Table 1). In our studies, dissociation of RAL and ELV from the Q148H/K/R substitutions was so fast that more than 50% of the $^3$H-labeled INI had already dissociated before the first time point at 20 min or an insufficient signal was obtained with $[^3H]$ELV, such that $k_{off}$ could not be measured (Q148K/R). By comparison with wild type, Y143C/H/R substitutions had the least effect on dissociation of DTG (1.2-, 1.6-, and 1.7-fold, respectively) and ELV (1.3-, 1.7-, and 1.6-fold, respectively) from the IN-DNA complexes (Fig. 3B; Table 1). In fact, for DTG the $k_{off}$ for the IN-DNA complex with the Y143C substitution may not be different from the $k_{off}$ for the wild-type IN-DNA complex ($P = 0.09$) (Fig. 4). The Y143 substitutions had a more substantial impact on dissociation of RAL from IN-DNA complexes, with 4.4-, 3.5-, and 8-fold increases in $k_{off}$ for Y143C/H/R, respectively (Table 1). The E92Q and G140S substitutions did impact the dissociation of all three INIs, but the dissociative half-life for DTG remained 17 to 20 h for these IN-DNA complexes (Table 2). The E138K substitution appeared to decrease the $k_{off}$ with all three INIs, but the difference may not be significant for DTG ($P = 0.1$).

### Impact of multiple IN substitutions on INI dissociation from IN-DNA complexes.

While single IN mutations are of interest as the first step in acquiring resistance, most frequently, multiple IN mutations develop during therapy with RAL and ELV (36). For RAL, a broad selection of secondary mutations occurs in the Q148 and N155 pathways (3), such as G140S or E138K with Q148H/K/R and E92Q with N155H. IN proteins with two substitutions, E92Q/N155H, E138K/Q148R, and G140S/Q148H, were prepared, and INI dissociation kinetics were determined. For DTG and RAL, INI dissociation from IN-DNA complexes containing double IN substitutions was faster ($P < 0.0003$) than dissociation from IN-DNA complexes containing the respective single IN substitution (Table 1; Fig. 4), except for Q148H and G140S/Q148H with RAL ($P = 0.76$). Despite the increase in $k_{off}$ for DTG, half of the $[^3H]$DTG remained bound to the IN-DNA complexes containing the double IN substitutions for at least 3.3 h, which was much longer than the half-life of RAL (0.2 to 0.3 h). Under the

### TABLE 1. INI off-rate from IN-DNA complexes at 37°C

| IN | $k_{off}$ ($s^{-1} \times 10^{-4}$) | Relative $k_{off}$ vs wild type |
|----|---------------------------------|--------------------------------|
|    | DTG | RAL | ELV | DTG | RAL | ELV |
| Wild type | 2.7 ± 0.4 | 22 ± 2 | 71 ± 4 | 1.0 | 1.0 | 1.0 |
| E92Q | 11.4 ± 0.3 | 59 ± 9 | 430 ± 20 | 4.2 | 2.7 | 6.1 |
| E138K | 2.3 ± 0.2 | 17 ± 0.3 | 52 ± 1 | 0.9 | 0.8 | 0.7 |
| G140S | 9.6 ± 0.8 | 44 ± 3 | 180 ± 20 | 3.6 | 2.0 | 2.5 |
| Y143C | 3.2 ± 0.1 | 96 ± 4 | 91 ± 2 | 1.2 | 4.4 | 1.3 |
| Y143H | 4.4 ± 0.2 | 78 ± 2 | 120 ± 6 | 1.6 | 3.5 | 1.7 |
| Y143R | 4.6 ± 0.3 | 176 ± 4 | 116 ± 5 | 1.7 | 8.0 | 1.6 |
| Q148H | 37 ± 3 | 1,160 ± 120 | 1,130 ± 140 | 13.7 | 53 | 16 |
| Q148K | 18 ± 5 | 730 ± 130 | ND | 6.7 | 33 | ND |
| Q148R | 21 ± 2 | 480 ± 80 | ND | 7.8 | 22 | ND |
| N155H | 20 ± 2 | 300 ± 80 | 500 ± 140 | 7.4 | 14 | 7.0 |
| E92Q/Q148H | 49 ± 3 | 770 ± 70 | ND | 18 | 35 | ND |
| E138K/Q148R | 53 ± 10 | 900 ± 340 | ND | 20 | 41 | ND |
| G140S/Q148H | 58 ± 8 | 1,130 ± 210 | ND | 22 | 51 | ND |

a $k_{off}$ values represent means and standard deviations for 3 to 8 independent experiments.

b ND, not determined due to low signal with $[^3H]$ELV.

c In vitro antiviral activity data (fold change in IN EC$_{50}$ for molecular clones with listed IN substitutions versus wild-type HIV-1) were previously reported (30).

d ND, not determined due to low signal with $[^3H]$ELV.
conditions used in these experiments, the signal with [3H]ELV was not sufficient to determine \( k_{\text{off}} \) for the double IN substitutions.

**Stability of IN-DNA complexes at 37°C.** For all IN-DNA-bead complexes where signal was generated from 3H-labeled INI binding, stable high signals were obtained for at least 12 days at 37°C (data not shown). To determine if incubation at 37°C impacted the enzymatic stability of the IN in the IN-DNA complex, strand transfer activity was monitored for IN-DNA complexes containing wild-type IN or the E92Q, Y143R, Q148H, N155H, E92Q/N155H, E138K/Q148R, or G140S/Q148H substitution(s). More than half (52 to 100%) of the initial (time = 1 h) strand transfer activity was observed for all IN-DNA complexes incubated for 24 h at 37°C, for all complexes except the E92Q/N155H IN-DNA complex (44%) incubated for at least 46 h, and for some complexes (N155H and G140S/Q148H) incubated for more than 70 h. For all tested IN-DNA complexes except the wild type, the INI dissociative half-lives were shorter than the time required for the loss of 50% of the strand transfer activity.

**Comparison of INI dissociation from IN-DNA complexes and antiviral potency.** In vitro antiviral studies have recently been reported for a broad panel of IN mutants, including mutants with the substitutions used in the dissociation experiments (30). In these studies, INI potency against HIV-1 isolates harboring site-directed NL432-based IN molecular clones was assayed in HeLa-CD4 cells and the fold change (FC) in the INI 50% effective concentration (EC50) versus that for wild-type HIV-1 was determined. For comparison purposes, the dissociative half-life values calculated from \( k_{\text{off}} \) and the published in vitro antiviral data are listed together in Table 2. In contrast, RAL dissociated quickly (\( t_{1/2} < 1 \) h) from the Q148H/K/R and N155H IN-DNA complexes and had decreased antiviral potency against wild-type virus harboring these substitutions (FCs, 8.4 to 83) (30). Consistent with the decreased antiviral potency for ELV with the Q148K/R and E92Q/N155H, E138K/Q148R, and G140S/Q148H substitutions (FCs, 240 to >1,700), increasing the concentration of [NH]ELV 4-fold in the dissociation experiments did not result in higher signals relative to background (data not shown), suggesting that these substitutions may reduce the binding affinity of ELV with these IN-DNA complexes. Overall, there is a qualitatively inverse relationship between the dissociative half-life and in vitro antiviral potency for DTG, RAL, and ELV (Fig. 5). This observation is consistent with results obtained with other INIs (13). Empirical observations based on our data suggest that in vitro resistance (FC in EC50 ≥3 versus wild type [30]) was not observed for IN-DNA complexes/INIs with a dissociative half-life of greater than 4 h and that pronounced...
vitro resistance was generally observed for IN-DNA complexes/INIs with a dissociative half-life of less than 1 h.

**DISCUSSION**

Our goal was to compare INI dissociation rates with wild-type and INI-resistant IN-DNA complexes for three INIs that have demonstrated efficacy in clinical studies. Included in this work were DTG (now in phase 3 clinical studies) and the two earlier INIs (RAL, the first FDA-approved INI, and ELV, which is also currently in phase 3 clinical studies). Resistance mutations observed in the clinic during treatment with RAL or ELV were used as a basis to select the IN substitutions for analysis, as no treatment-naïve subject has yet developed resistance to DTG.

For RAL, two main pathways for resistance involve signature mutations which are initially observed at amino acids Q148 and N155 and are almost invariably found with secondary mutations which may increase resistance and impact viral fitness and catalytic efficiency (5, 11, 18, 38, 40). With additional viral replication, mutations may be selected at Y143C and Y143R (12, 44), which then may outcompete the original mutant viruses. Studies have shown that the Y143, Q148, and N155 primary mutations or the addition of Q148 and N155 pathway secondary mutations has less impact on the in vitro enzyme and antiviral potencies of DTG than those of RAL and ELV (25, 30, 48). In our experiments, DTG demonstrated significantly slower dissociation from all IN-DNA complexes than RAL and ELV. The Q148H substitution caused the greatest fold increase in $k_{	ext{off}}$ for all three INIs. While the addition of the Q148 and N155 secondary mutations did cause an additional increase in $k_{	ext{off}}$ for DTG compared to the primary substitutions, the effect was less pronounced than it was for RAL. DTG maintained prolonged binding even with the IN-DNA complex containing G140S and Q148H, mutations that are frequently observed in RAL resistance during treatment (36) but have limited impact on the in vitro potency of DTG (25, 30). The in vitro biochemical and antiviral data generated with DTG suggest that an accumulation of IN mutations may be required in these RAL signature resistance pathways to have effects on DTG binding and potency similar to those observed for RAL and ELV.

The crystal structures of prototype foamy virus (PFV) IN in complex with DNA and RAL, ELV, (24), or DTG (25) and HIV-1 IN structural modeling studies (8, 9) provide insight into INI binding modes and how substitutions in the active site could impact INI binding. For RAL, the co-crystal structures show a key π-stacking interaction between RAL’s oxadiazole and the side chain of Y212 in PFV IN, which corresponds to Y143 in HIV-1 IN. Since substitution of Y143 with His, Cys, or Arg likely compromises this interaction, RAL’s dissociation rates are expected to increase, with the Y143R substitution having the greatest effect, given its flexibility and formal charge. The co-crystal structures with ELV and DTG reveal that these INIs make only limited van der Waals contact with Y143, suggesting that ELV and DTG dissociation rates would be minimally impacted by Y143 substitutions, which is consistent with our dissociation results. From a comparison of the PFV IN-DNA structures, the S217H and N224H (Q148H and N155H, respectively, in HIV-1 IN) substitutions appear to alter the architecture of the structural and catalytic components of the IN active site, which, moreover, appear to perturb the binding of the INIs. We speculate that Q148 may play a role in stabilizing the HIV-1 IN active-site loop into a catalytically active state and that His, Lys, or Arg substitutions at this position may alter the loop, perhaps to a greater extent than is evident in the PFV IN structures, given the added flexibility of the HIV-1 IN loop imparted by G140 (8). Also, the N155H substitution may alter the base of the HIV-1 IN catalytic pocket, the placement of at least the Mg$^{2+}$ ion coordinated to E152, and the structure of that portion of the α4 helix forming one side of the pocket, causing a minor displacement of the INIs within the pocket. Altogether, these changes would be expected to increase $k_{	ext{off}}$ for all three INIs, which is in fact observed. Consistent with our findings, an altered loop configuration or a displacement of the coordination complex would be expected to have the greatest negative impact on RAL binding, given the possibility of disrupting its π-stacking interaction with Y143. Additionally, the structural (8) and electronic (9) characteristics of DTG’s metal-binding scaffold may contribute to the slower dissociation kinetics of DTG than RAL and ELV. Overall, the dissociation data are consistent with the crystallography and structural modeling results and suggest that key aspects of both the IN active site and the INIs may contribute to the observed differences in INI dissociation rates from the IN-DNA complexes.

The dissociation rate of a drug from its target typically is a major component of residence time, which can be defined as the period during which the ligand (drug) is bound to its receptor (6). A longer residence time on the targeted receptor theoretically provides beneficial characteristics; this is because the ligand has a greater opportunity to have an effect. However, the relationship between in vitro measurements of dissociation and in vivo efficacy is often qualitative rather than quantitative; ligand and receptor effective concentrations may vary substantially during the course of dosing and as a function of ongoing biological processes. As such, evaluation of the in vitro and in vivo relationship should take into account the window or duration of the pharmacological effect. In the case of HIV, there is a window of opportunity for many processes, including those targeted by current anti-HIV agents. For IN strand transfer inhibitors such as the INIs studied here, the window during which binding most likely occurs is between 3’ processing (which generates structural components of the catalytic binding pocket [15, 26] of the HIV cDNA genome ends) and integration into the host genome. Interestingly, in vitro washout experiments found an association between an extended INI dissociative half-life and a more persistent antiviral effect, apparently via the irreversible generation of unintegrated viral cDNA (13). The dissociation rate of INIs from IN-DNA complexes in vitro, which should be reflected in the residence time, might also be an important component of INI efficacy in vivo.

An important consideration is that prolonged binding of DTG to IN-DNA complexes with INI resistance mutations would be suggestive of a higher barrier to resistance. Since INI binding generates nonreplicative forms of unintegrated viral cDNA, such as 2 long terminal repeats (27), there is less opportunity for the virus to undergo further rounds of replication which can generate additional mutations and thereby
lead to higher levels of resistance. In the case where these mutations are the first step in a sequence of mutations required to achieve high-level resistance, prolonged INI binding theoretically should provide an improved potential for a higher barrier to resistance.

The comparison of dissociative half-life and antiviral potency suggests that there might be a threshold residence time for INIs which impacts antiviral efficacy in the cell-based system used to determine antiviral potency (30). From our data set, this threshold would be a dissociative half-life of between 1 and 4 h, but additional data would be needed to further refine this relationship and to identify exceptions. It is possible that the threshold is related to the length of the window during HIV replication when IN activity is required. One could speculate that, in particular during periods of nonadherence when drug levels may reach suboptimal levels, an INI with a longer dissociative half-life might remain bound and prevent integration. Additional experiments with systems in which this integration window is significantly longer, such as primary blood-derived monocytes, might be illuminating. In addition, time-of-removal or time-of-addition experiments might reveal whether DTG has a prolonged antiviral effect upon washout similar to what was observed for BMS-878397 compared to RAL (13).

The dissociation data presented here provide additional evidence of differential binding of INIs to wild-type and mutant IN-DNA complexes and suggest that prolonged INI binding may contribute to efficacy. While dissociation kinetics and compound residence time are likely to be important for INIs, other factors such as the rate of compound association (48), drug pharmacokinetics, and viral fitness could also impact efficacy and resistance. Additional work is necessary to elucidate the contribution of each of these components to the emergence of INI resistance. However, DTG’s long dissociation half-life with the wild-type and INI-resistant IN-DNA complexes may contribute to its distinct resistance profile and highlight the potential for improved activity against wild-type HIV-1 and clinically relevant INI-resistant viruses. Among the potential important predictions from the preceding discussion is that DTG might have a higher capacity to suppress viral replication should drug levels become suboptimal due to adherence problems (i.e., possess “forgiveness”) and that the slower dissociation may translate to a higher genetic barrier to resistance in vivo. Ultimately, however, such predictions will need to be verified by data from the phase 2b and phase 3 clinical studies which are currently ongoing.

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REFERENCES

1. Asante-Appiah, E., and A. M. Skalka. 1999. HIV-1 integrase: structural organization, conformational changes, and catalysis. Adv. Virus Res. 52:351–369.
2. Boros, E. E., B. A. Johns, E. P. Garvey, C. S. Kohle, and W. H. Miller. 2006. Synthesis and HIV-integrase strand transfer inhibition activity of 7-hydroxy[1,3]thiazolo[5,4-b]pyridin-5(4H)-ones. Bioorg. Med. Chem. Lett. 16:5665–5672.
3. Ceccherini-Silberstein, F., et al. 2009. Characterization and structural analysis of HIV-1 integrase core domain. J. Virol. 83:1–20.
4. Chin, T. K., and D. R. Davies. 2004. Structure and function of HIV-1 integrase. Curr. Top. Med. Chem. 4:965–977.
5. Cooper, D. A., et al. 2008. Subgroup and resistance analyses of raltegravir for resistant HIV-1 infection. N. Engl. J. Med. 359:355–365.
6. Copeland, R. A., D. L. Pompliano, and T. D. Meek. 2006. Drug-target residence time and its implications for lead optimization. Nat. Rev. Drug Discov. 5:730–739.
7. Craige, R. 2001. HIV integrase, a brief overview from chemistry to therapeutics. J. Biol. Chem. 276:2213–2216.
8. DeAnda, F., K. Hattori, T. Yoshinaga, T. Kawasuji, and M. R. Underwood. 2010. Structural models of HIV-1 integrase and DNA in complex with S/GSK1349572, raltegravir, or elvitegravir: structural-based rationale for INI resistance profiles. Antiviral Res. 86(Suppl. 2):A73.
9. DeJesus, E., et al. 2007. First report of raltegravir (RAL, MK-0518) use after viriologic rebound on elvitegravir (EVT, GS 9137), abstr. TUPEB052. Abstr. 39th Intersc. Conf. Antimicrob. Agents Chemother. American Society for Microbiology, Washington, DC.
10. DeJesus, E., et al. 2007. First report of raltegravir (RAL, MK-0518) use after viriologic rebound on elvitegravir (EVT, GS 9137), abstr. TUPEB052. Abstr. 39th Intersc. Conf. Antimicrob. Agents Chemother. American Society for Microbiology, Washington, DC.
11. DeJesus, E., et al. 2007. First report of raltegravir (RAL, MK-0518) use after viriologic rebound on elvitegravir (EVT, GS 9137), abstr. TUPEB052. Abstr. 39th Intersc. Conf. Antimicrob. Agents Chemother. American Society for Microbiology, Washington, DC.
12. DeJesus, E., et al. 2007. First report of raltegravir (RAL, MK-0518) use after viriologic rebound on elvitegravir (EVT, GS 9137), abstr. TUPEB052. Abstr. 39th Intersc. Conf. Antimicrob. Agents Chemother. American Society for Microbiology, Washington, DC.
13. Dicker, I. B., et al. 2011. Longier integrase inhibitor dissociation half-lives induce persistent anti-HIV effects in cell culture, abstr. 521. 18th Int. Conf. Retrovir. Opportunistic Infect.
14. Dicker, I. B., et al. 2008. Biochemical analysis of HIV-1 integrase variants susceptible to strand transfer inhibitors. J. Biol. Chem. 283:23599–23609.
15. Dicker, I. B., et al. 2007. Changes to the HIV long terminal repeat and to HIV integrase differentially impact HIV integrase assembly, activity, and the binding of strand transfer inhibitors. J. Biol. Chem. 282:31386–31396.
16. Eron, J., et al. 2010. Activity of the integrase inhibitor S/GSK1349572 in subjects with HIV exhibiting raltegravir resistance: week 24 results of the VIKING study (ING112961). J. Int. AIDS Soc. 13:E51.
17. Esposito, D., and R. Craigie. 1999. HIV integrase structure and function. Adv. Virus Res. 52:219–333.
18. Fransen, S., et al. 2009. Loss of raltegravir susceptibility by human immuno deficiency virus type 1 is conferred via multiple nonoverlapping genetic pathways. J. Virol. 83:11440–11446.
19. Garvey, E. P., et al. 2009. Potent inhibitors of HIV-1 integrase display a two-step, slow-binding inhibition mechanism, which is absent in a drug-resistant T66I/M154I mutant. Biochemistry 48:1644–1653.
20. Goethals, O., et al. 2010. Primary mutations selected in vitro with raltegravir confer large fold changes in susceptibility to first-generation integrase inhibitors, but minor fold changes to inhibitors with second-generation resistance profiles. Virology 402:338–346.
21. Goethals, O., et al. 2008. Resistance mutations in human immunodeficiency virus type 1 integrase selected with elvitegravir confer reduced susceptibility over a wide range of integrase inhibitors. J. Virol. 82:10366–10374.
22. Grobler, J. A., K. A. Stillmock, and D. J. Hazuda. 2009. Scintillation proximity assays for mechanistic and pharmacological analyses of HIV-1 integrase inhibition. Methods 47:249–253.
23. Grobler, J. A., et al. 2009. Functionally irreversible inhibition of integration by slowly dissociating strand transfer inhibitors, abstr. O-10. Abstr. 10th Int. Workshop Clin. Pharmacol. HIV Ther.
24. Hare, S., et al. 2010. Molecular mechanisms of retroviral integrase inhibition and the evolution of viral resistance. Proc. Natl. Acad. Sci. U. S. A. 107:20057–20062.
25. Hare, S., et al. 30 June 2011, posting date. Structural and functional analyses of the second-generation integrase strand transfer inhibitor dolutegravir (S/GSK1349572). Mol. Pharmacol. doi:10.1124/mol.111.073189.
26. Hare, S., S. S. Gupta, E. Valkov, A. Engelman, and P. Cherepanov. 2010. Retrovirial intasome assembly and inhibition of DNA strand transfer. Nature 464:232–236.
27. Hazuda, D. J., et al. 2000. Inhibitors of strand transfer that prevent integration and inhibit HIV-1 replication in cells. Science 287:646–650.
28. Hluhanich, R., et al. 2010. HIV integrase inhibitors (INIs) do not exert a post-antibiotic effect (PAE) despite slow dissociation from IN-DNA complexes in vitro, abstr. H-930. Abstr. 49th Intersci. Conf. Antimicrob. Agents Chemother. American Society for Microbiology, Washington, DC.
29. Johns, B., et al. 2010. The discovery of S/GSK1349572: a once-daily next generation integrase inhibitor with a superior resistance profile, abstr. 55. Abstr. 17th Int. Conf. Retrovir. Opportunistic Infect.
30. Kobayashi, M., et al. 2011. Evolutionary virology of S/GSK1349572, a next-generation HIV integrase inhibitor. Antimicrob. Agents Chemother. 55:813–821.
31. LaFemina, R. L., et al. 1992. Requirement of active human immunodeficiency virus type 1 integrase enzyme for productive infection of human T-lymphoid cells. J. Virol. 66:7414–7419.

32. Langley, D. R., et al. 2008. The terminal (catalytic) adenosine of the HIV LTR controls the kinetics of binding and dissociation of HIV integrase strand transfer inhibitors. Biochemistry 47:13481–13488.

33. Lewinski, M. K., and F. D. Bushman. 2005. Retroviral DNA integration—mechanism and consequences. Adv. Genet. 55:147–181.

34. Liao, C., C. Marchand, T. R. Burke, Jr., Y. Pommier, and M. C. Nicklaus. 2010. Authentic HIV-1 integrase inhibitors. Future Med. Chem. 2:1107–1122.

35. Markowitz, M., et al. 2007. Rapid and durable antiretroviral effect of the HIV-1 integrase inhibitor raltegravir as part of combination therapy in treatment-naive patients with HIV-1 infection: results of a 48-week controlled study. J. Acquir. Immune Defic. Syndr. 46:125–133.

36. McColl, D. J., and X. Chen. 2010. Strand transfer inhibitors of HIV-1 integrase: bringing IN a new era of antiretroviral therapy. Antiviral Res. 85:101–118.

37. Métifiot, M., C. Marchand, K. Maddali, and Y. Pommier. 2010. Resistance to integrase inhibitors. Viruses 2:1347–1366.

38. Métifiot, M., et al. 2010. Biochemical and pharmacological analyses of HIV-1 integrase flexible loop mutants resistant to raltegravir. Biochemistry 49:3715–3722.

39. Min, S., et al. 29 June 2011, posting date. Antiviral activity, safety, and pharmacokinetics/pharmacodynamics of dolutegravir as 10-day monotherapy in HIV-1-infected adults. AIDS doi:10.1097/QAD.0b013e32834a1dd9.

40. Nakahara, K., et al. 2009. Secondary mutations in viruses resistant to HIV-1 integrase inhibitors that restore viral infectivity and replication kinetics. Antiviral Res. 81:141–146.

41. Ratner, L., et al. 1985. Complete nucleotide sequence of the AIDS virus, HTLV-III. Nature 313:277–284.

42. Rockstroh, J., et al. 2010. Once-daily S/GSK1349572 combination therapy in antiretroviral-naïve adults: rapid and potent 24-week antiviral responses in SPRING-1 (ING112276). J. Int. AIDS Soc. 13:OS0.

43. Shimura, K., et al. 2008. Broad antiretroviral activity and resistance profile of the novel human immunodeficiency virus integrase inhibitor elvitegravir (JTK-303/GS-9137). J. Virol. 82:764–774.

44. Shin, C. G., B. Taddeo, W. A. Haseltine, and C. M. Farnet. 1994. Genetic analysis of the human immunodeficiency virus type 1 integrase protein. J. Virol. 68:1633–1642.

45. Steigbigel, R. T., et al. 2008. Raltegravir with optimized background therapy for resistant HIV-1 infection. N. Engl. J. Med. 359:339–354.

46. Underwood, M. R., B. Johns, A. Sato, T. Fujiwara, and W. Spreen. 2009. S/GSK1349572: a next generation integrase inhibitor with activity against integrase inhibitor-resistant clinical isolates from patients experiencing virologic failure while on raltegravir therapy, abstr. WEPEA098. Abstr. 5th Int. AIDS Soc. Conf. HIV Pathogenesis, Treatment, Prevention. International AIDS Society, Geneva, Switzerland.

47. Witmer, M., and R. Danovich. 2009. Selection and analysis of HIV-1 integrase strand transfer inhibitor resistant mutant viruses. Methods 47:277–282.

48. Yoshinaga, T., M. Kanamori-Koyama, T. Seki, A. Sato, and T. Fujiwara. 2010. Strong inhibition of wild-type and integrase inhibitor (INI)-resistant HIV integrase (IN) strand transfer reaction by the novel INI S/GSK1349572. Antivir. Ther. 15(Suppl. 2):A12.