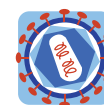# In vitro activity of dolutegravir against wild-type and integrase inhibitor-resistant HIV-2

Smith *et al.*

RETROVIROLOGY

SHORT REPORT

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In vitro activity of dolutegravir against wild-type and integrase inhibitor-resistant HIV-2

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Abstract

Background: Dolutegravir recently became the third integrase strand transfer inhibitor (INSTI) approved for use in HIV-1–infected individuals. In contrast to the extensive dataset for HIV-1, in vitro studies and clinical reports of dolutegravir for HIV-2 are limited. To evaluate the potential role of dolutegravir in HIV-2 treatment, we compared the susceptibilities of wild-type and INSTI-resistant HIV-1 and HIV-2 strains to the drug using single-cycle assays, spreading infections of immortalized T cells, and site-directed mutagenesis.

Findings: HIV-2 group A, HIV-2 group B, and HIV-1 isolates from INSTI-naïve individuals were comparably sensitive to dolutegravir in the single-cycle assay (mean EC_{50} values = 1.9, 2.6, and 1.3 nM, respectively). Integrase substitutions E92Q, Y143C, E92Q + Y143C, and Q148R conferred relatively low levels of resistance to dolutegravir in HIV-2$_{ROD9}$ (2- to 6-fold), but Q148K, E92Q + N155H, T97A + N155H and G140S + Q148R resulted in moderate resistance (10- to 46-fold), and the combination of T97A + Y143C in HIV-2$_{ROD9}$ conferred high-level resistance (>5000-fold). In contrast, HIV-1$_{NL4-3}$ mutants E92Q + N155H, G140S + Q148R, and T97A + Y143C showed 2-fold, 4-fold, and no increase in EC_{50}, respectively, relative to the parental strain. The resistance phenotypes for E92Q + N155H, and G140S + Q148R HIV-2$_{ROD9}$ were also confirmed in spreading infections of CEM-ss cells.

Conclusions: Our data support the use of dolutegravir in INSTI-naïve HIV-2 patients but suggest that, relative to HIV-1, a broader array of replacements in HIV-2 integrase may enable cross-resistance between dolutegravir and other INSTI. Clinical studies are needed to evaluate the efficacy of dolutegravir in HIV-2–infected individuals, including patients previously treated with raltegravir or elvitegravir.

Findings

Human immunodeficiency virus type 2 (HIV-2) infection is a significant public health problem in West Africa and has been reported in other countries with socioeconomic ties to the region [1]. Dual HIV-1/HIV-2 infection also occurs in areas where the viruses co-circulate [2-6]. Historically, clinical outcomes of antiretroviral therapy in HIV-2 and HIV-1/HIV-2 dually positive patients have been poor, with high rates of immuno-virologic failure and emergent multidrug resistance [7-11]. Newer classes of antiretrovirals (ARV) with anti–HIV-2 activity could represent substantial improvements to the current therapeutic picture [12,13].

A growing body of evidence suggests that integrase strand transfer inhibitors (INSTI) might be particularly useful for HIV-2 treatment. Raltegravir and elvitegravir are both potent inhibitors of HIV-2 replication in culture [14-18], and case reports and small case series (primarily involving ARV-experienced individuals) indicate that raltegravir and elvitegravir can reduce HIV-2 viral loads when combined with other suppressive ARV [19-32]. As with HIV-1, changes at integrase residues Y143, Q148 or N155, together with other secondary replacements in the integrase protein (*i.e.*, E92Q, T97A, G140S, and possibly others), confer resistance to raltegravir in HIV-2 [26,28-35]. The emergence of resistance to elvitegravir has not yet been reported in HIV-2-infected individuals but will likely involve these same three pathways based on studies of HIV-1 [36-50] and the extensive cross-resistance seen between raltegravir and elvitegravir in HIV-2 in culture [15,17]. Clinical trials of raltegravir- and

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elvitegravir-containing regimens for first-line HIV-2 treatment are now underway and are expected to yield data within the next few years (NCT01605890, NCT02150993, NCT02180438).

A third strand transfer inhibitor, dolutegravir, was recently approved by the United States Food and Drug Administration (FDA) for use in both INSTI-naïve and INSTI-experienced HIV-1 patients. Although dolutegravir has been extensively evaluated for HIV-1 treatment, few studies have examined its potential use in HIV-2–infected individuals. Charpentier and colleagues reported that HIV-2$_{ROD,}$ HIV-1$_{BRU}$, and eight HIV-2 isolates from INSTI-naïve patients were comparably susceptible to dolutegravir in spreading infections of peripheral blood mononuclear cells (PBMC) (EC$_{50}$ = 0.2–4 nM) and that three HIV-2 isolates from raltegravir-treated individuals with consensus integrase genotypes G140S + Q148R (group A), G140T + Q148R + N155H (group A), and T97A + Y143C (group H) were 63-, 9-, and 5-fold resistant to dolutegravir, respectively, in PBMC [51]. In addition, the manufacturer of dolutegravir (ViiV Healthcare) reported that EC$_{50}$ values against three clinical isolates of HIV-2 ranged from 0.09 nM to 0.61 nM in PBMC assays, and that combinations of substitutions A153G + N155H + S163G and E92Q + T97A + N155H + S163D in HIV-2 integrase conferred 4-fold decreases in dolutegravir susceptibility, while E92Q + N155H and G140S + Q148R resulted in 8.5-fold and 17-fold decreases, respectively [52].

The ability of dolutegravir to inhibit strains resistant to other INSTI is of particular importance–in HIV-1, mutations Q148H/K/R, together with secondary changes in the integrase protein, confer resistance to dolutegravir in cell culture [38,47,53-55], and other mutations associated with diminished in vitro susceptibility to dolutegravir have been reported [56-61]. In contrast, dolutegravir is fully active against HIV-1 variants bearing Y143 or N155 mutations (with or without secondary changes) in both single-cycle and spreading infection assays [38,47,53-55], although it should be noted that Y143 and N155 mutants have been observed in raltegravir-experienced patients who subsequently failed dolutegravir-based regimens [62,63]. In the VIKING-3 trial, dolutegravir response rates (<50 HIV-1 RNA copies/ml at week 24) declined from 79% (n = 100/126) for patients without Q148 mutations at baseline (including those with N155H, Y143C/H/R, T66A, E92Q, or historical evidence of INSTI resistance), to 58% (21/36) for patients with Q148 plus one additional secondary mutation, to 24% (5/21) for those with Q148 plus two or more secondary mutations [64]. Importantly, drug resistance testing is not widely available in West Africa, and thus, dolutegravir usage in many HIV-2–infected patients, including INSTI-experienced individuals, will depend on an algorithmic approach to treatment. To date, there are only two reports of dolutegravir treatment for

HIV-2 infection ([65,66]; n = 2 and 13 patients, respectively), with limited duration of follow-up.

In the present study, we examined the activity of dolutegravir against wild-type and INSTI-resistant HIV-2 strains using an indicator cell assay that restricts viral replication to a single cycle [15]. This methodology enables a direct comparison of HIV-1 and HIV-2 drug susceptibility while avoiding potential confounders such as differences in replication rates, infectivity, cytopathic potential and cell-to-cell spread.

We initially compared the dolutegravir sensitivities of viruses derived from two prototypic full-length molecular clones: pNL4-3 (HIV-1 group M, subtype B) and pROD9 (HIV-2 group A). In head-to-head single-cycle assays, these two strains showed nearly identical dose-response profiles (Figure 1A). Over multiple assays runs, the mean EC$_{50}$ values for dolutegravir (± standard deviation) were 1.5 ± 0.6 nM for HIV-1$_{NL4-3}$ and 2.3 ± 0.7 nM for HIV-2$_{ROD9}$ (n = 14 and 24 determinations, respectively). Dolutegravir was 3.6-fold more potent than raltegravir and 9.1-fold more potent than elvitegravir against HIV-2$_{ROD9}$ (Figure 1B). Other isolates from ARV-naïve individuals displayed levels of dolutegravir sensitivity comparable to HIV-1$_{NL4-3}$ and HIV-2$_{ROD9}$ (Figure 1C). The aggregate EC$_{50}$ values for HIV-1, HIV-2 group A, and HIV-2 group B were 1.3 ± 0.2 nM, 1.9 ± 0.5 nM, and 2.6 ± 0.9 nM, respectively. When subjected to a one-way ANOVA, only the comparison between HIV-1 and HIV-2 group B reached statistical significance (p < 0.05); this modest difference was attributable to the slightly higher EC$_{50}$ for HIV-2$_{EHO}$ (3.6 ± 1.9 nM) (Figure 1C). Notably, HIV-2$_{EHO}$ integrase contains a glutamate at position 146, whereas other HIV-2 isolates (as well as HIV-1) encode glutamine at this site [67,68]. Substitutions at Q146 have been observed in HIV-1 following in vitro selections with elvitegravir and other, investigational INSTI [18,69,70]. To our knowledge, Q146 mutations have not been observed in HIV-2 variants selected in culture, nor have they been reported in HIV-2 patients treated with INSTI-based regimens.

To examine potential resistance pathways in HIV-2, we tested the activity of dolutegravir against a panel of site-directed mutants of HIV-2$_{ROD9}$ using the single-cycle assay. These variants contained amino acid replacements in the integrase protein that are associated with raltegravir and elvitegravir treatment; their phenotypes with respect to raltegravir and elvitegravir susceptibility have been reported elsewhere [14,15]. Single amino acid changes T97A, G140S, Q148H and N155H had no significant effect on dolutegravir sensitivity (p > 0.05, ANOVA; Figure 2A). In contrast, mutants E92Q, Y143C, E92Q + Y143C, Q148K, and Q148R were resistant to dolutegravir, with EC$_{50}$ values 2.3–9.3-fold greater than that of the parental strain (Figure 2A), and variants
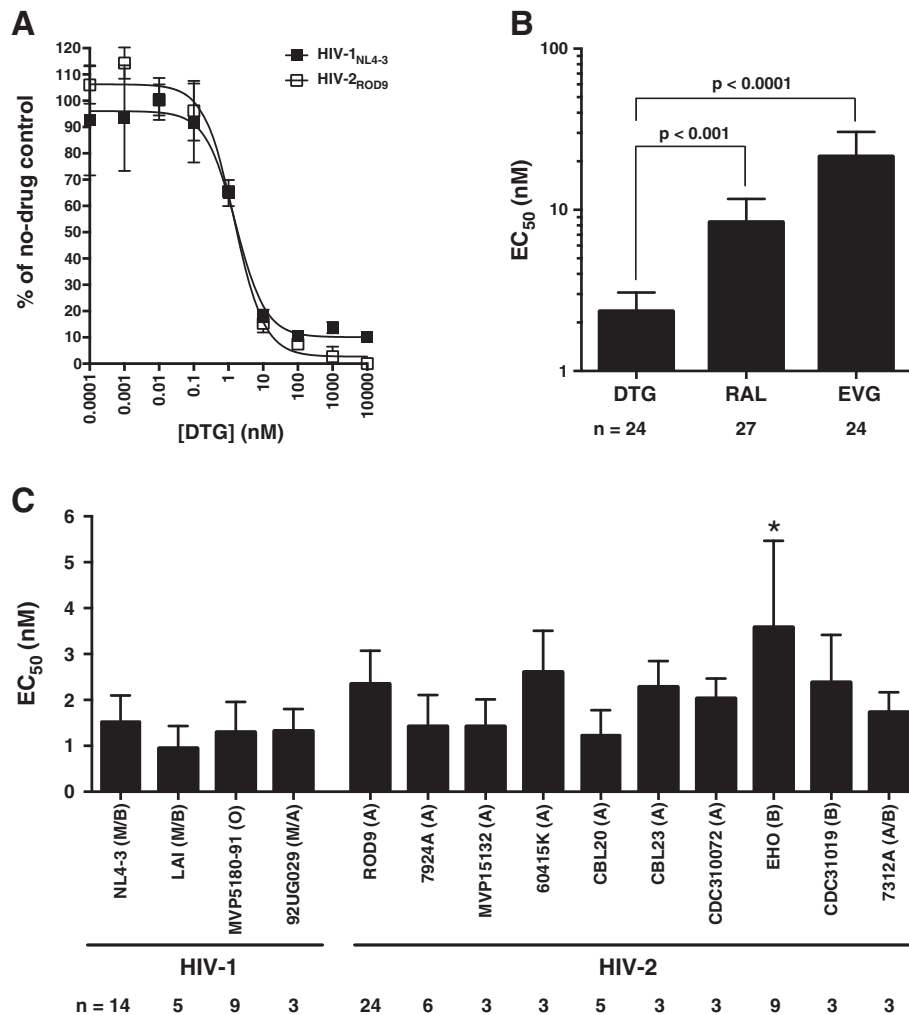
Figure 1 Susceptibility of wild-type HIV-1 and HIV-2 isolates to dolutegravir in the single-cycle assay. (A) Representative dose-response profiles for HIV-1$_{NL4-3}$ and HIV-2$_{ROD9}$. Virus stocks were generated by transient transfection of chloroquine-treated 293T/17 cultures with plasmids pNL4-3 and pROD9, respectively. Dolutegravir was obtained from Selleck Chemicals, Inc. Titers are expressed as the percentage of no-drug (solvent-only) controls and are the means of two independent cultures at each drug concentration. Curve fits were generated using the sigmoid dose-response function of Prism version 6.0 (GraphPad Software, Inc.). **(B)** Comparison of the activity of dolutegravir (DTG), raltegravir (RAL), and elvitegravir (EVG) against wild-type HIV-2$_{ROD9}$. Values for RAL and EVG include data from two previously-published studies of HIV-2 from our group [14,15] plus additional determinations; all data were obtained using the single-cycle assay. Bars indicate mean 50% effective concentrations (EC$_{50}$); the number of independent determinations (n) for each strain is shown below the x-axis. P values were obtained via analysis of variance (ANOVA) of log$_{10}$-transformed EC$_{50}$ values with Tukey's post test (Prism v6.0). No cytotoxic effects were observed in dolutegravir-treated MAGIC-5A cultures at concentrations as high as 10,000 nM. **(C)** Activity of dolutegravir against wild-type HIV-1 and HIV-2 isolates. Group/subtype designations are shown in parentheses. HIV-1$_{NL4-3}$ and HIV-2$_{ROD9}$ were generated as in panel A. HIV-2$_{EHO}$ was kindly provided by Jan McClure (University of Washington). The remaining isolates were obtained from the National Institutes of Health AIDS Reagent Program (www.aidsreagent.org). *, significantly greater than HIV-2$_{ROD9}$, HIV-2$_{MVP15132}$, HIV-2$_{CBL20}$, and all HIV-1 isolates (p < 0.05, ANOVA with Tukey's post test). In all panels, error bars indicate standard deviations.

E92Q + N155H, T97A + N155H and G140S + Q148R exhibited 11–33-fold resistance to the drug (p < 0.05, ANOVA; Figure 2A and B). In experiments with T97A + Y143C HIV-2$_{ROD9}$, dolutegravir concentrations as high as 10 μM failed to reduce viral replication by 50% (Figure 2A and C; EC$_{50}$ > 10 μM), although modest dose-dependent inhibition was apparent at doses ≥100 nM (Figure 2C).

Altogether, nine of the 13 HIV-2 integrase mutants tested were resistant to dolutegravir in the single-cycle assay (Figure 2A).

We also evaluated the dolutegravir sensitivities of E92Q + N155H, T97A + Y143C, and G140S + Q148R HIV-2$_{ROD9}$ in three-day spreading infections of immortalized T cells (CEM-ss). These assays were preformed as previously
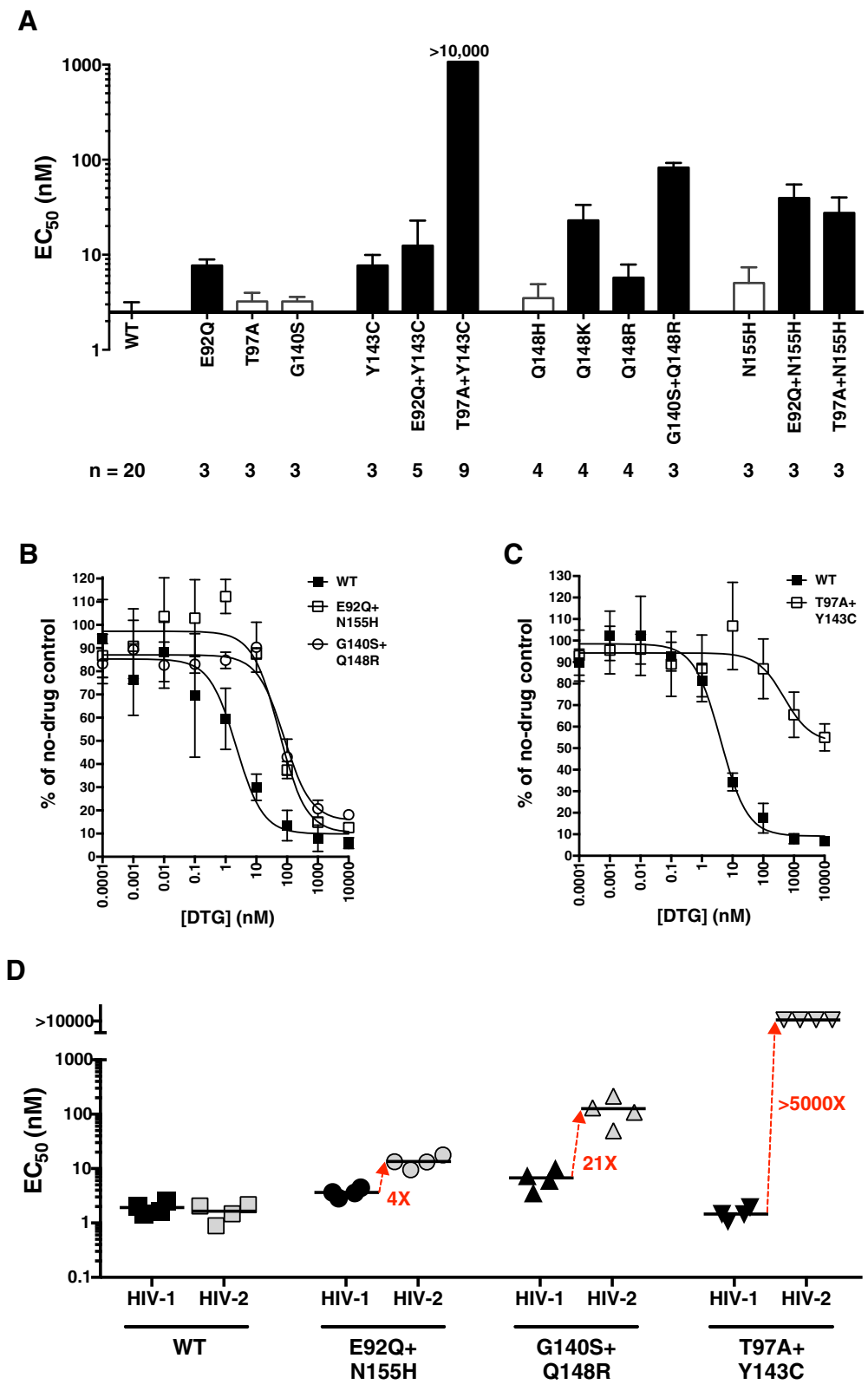
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Figure 2 Antiviral activity of dolutegravir against site-directed mutants of HIV-1 and HIV-2 integrase in the single-cycle assay. (A) EC_{50} values for wild-type (WT) HIV-2$_{ROD9}$ and HIV-2$_{ROD9}$ integrase mutants generated in the full-length pROD9 molecular clone. Shaded bars indicate strains that are significantly different from wild-type ($p < 0.05$, ANOVA of $\log_{10}$-transformed EC_{50} values with Tukey's post test; Prism v6.0). The number of independent determinations (n) for each strain is shown below the x-axis. **(B)** and **(C)** Representative dose-response profiles for WT, E92Q + N155H, G140S + Q148R and T97A + Y143C HIV-2$_{ROD9}$. Data are the means of three independent cultures at each dose of dolutegravir (DTG). Curve fits were generated as described in Figure 1A. **(D)** Comparative analysis of equivalent site-directed mutants of HIV-1 and HIV-2 integrase. Each point is the result of a single dose-response assay performed as described in Figure 1A. Horizontal bars indicate the mean EC_{50} values for each strain. Red arrows and text indicate fold increases in the mean EC_{50} values for HIV-2 relative to HIV-1. ANOVA results for these data are described in the main text. In all panels, error bars represent standard deviations.

described for the MT-2 T cell line [14]. The resultant EC_{50} values for the parental strain, E92Q + N115H, and G140S + Q148R were 0.24, 2.1 and 73 nM, respectively, indicating 8.8-fold resistance to dolutegravir for E92Q + N115H and 300-fold resistance for G140S + Q148R. Despite repeated attempts using high multiplicities of infection (≥0.1) and prolonged incubation times (up to seven days), CEM-ss cultures inoculated with T97A + Y143C HIV-2$_{ROD9}$ failed to produce detectable levels of infectious virus, indicating a severe fitness defect. This result is consistent with the poor replication capacity previously reported for T97A + Y143C HIV-2$_{ROD9}$ [15].

Lastly, we performed a head-to-head comparison of the phenotypes conferred by E92Q + N155H, G140S + Q148R, and T97A + Y143C in HIV-1$_{NL4-3}$ and HIV-2$_{ROD9}$ in the single-cycle assay. G140S + Q148R resulted in slight resistance to dolutegravir in HIV-1$_{NL4-3}$ (3.5-fold; p <0.01, ANOVA), whereas E92Q + N155H and T97A + Y143C had no statistically significant effect in the HIV-1$_{NL4-3}$ background (Figure 2D). These data are entirely consistent with previous studies of HIV-1 [38,47,53,54]. In contrast, HIV-2$_{ROD9}$ mutants E92Q + N155H, G140S + Q148R, and T97A + Y143C were all resistant to dolutegravir (p < 0.0001, ANOVA) and showed EC_{50} values 4-, 21- and >5000-fold greater than those seen for equivalent mutants of HIV-1$_{NL4-3}$, respectively (Figure 2D). EC_{50} and fold change values for all HIV-1$_{NL4-3}$ and HIV-2$_{ROD9}$ integrase mutants tested in this study, together with the corresponding EC_{50} values for the parental wild-type clones, are compiled in Table 1.

Taken together, our results indicate that prototypic HIV-1 and HIV-2 strains, as well as HIV-1 and HIV-2 isolates from INSTI-naïve individuals, are comparably sensitive to dolutegravir in a single cycle of viral replication in MAGIC-5A indicator cells (Figure 1). These findings complement previous data from spreading infections of PBMC [51]–using a different methodology and target cell type–and suggest that dolutegravir would be an appropriate treatment choice for INSTI-naïve HIV-2 patients when combined with other HIV-2–active ARV. We also report the effects of raltegravir-associated mutations on dolutegravir susceptibility using site-directed mutagenesis of genetically-defined HIV-1 and HIV-2 molecular clones (pNL4-3 and pROD9, respectively).

Our analysis shows that equivalent amino acid changes in the integrase proteins of HIV-1 and HIV-2 can have differing effects on dolutegravir susceptibility (Figure 2D) and that, in HIV-2$_{ROD9}$, integrase changes Q148K, T97A + Y143C, E92Q + N155H, T97A + N155H, and G140S + Q148R confer moderate to high levels of dolutegravir resistance (≥10-fold; Figure 2A–C and Table 1). We cannot exclude the possibility that the resistance levels observed in our site-directed HIV-2 mutants are specific to the ROD9 molecular clone, as the genetic context within integrase can have a substantial impact on the phenotypic expression of INSTI resistance [71,72]. For example, in the aforementioned study by Charpentier et al.

Table 1 Compilation of EC_{50} and fold change values for site-directed mutants of HIV-2$_{ROD9}$ and HIV-1$_{NL4-3}$ integrase

HIV Type	Strain	EC_{50} for DTG (nM)[a]	n[b]	Fold Change[c]
HIV-2	Wild-type	2.3 ± 0.7	24	
	E92Q	**7.7 ± 1.2**	3	3
	T97A	3.2 ± 0.8	3	1
	G140S	3.2 ± 0.8	3	1
	Y143C	**7.7 ± 2.2**	3	3
	Q148H	3.5 ± 1.4	4	1
	Q148K	**23 ± 10**	4	10
	Q148R	**5.7 ± 2.1**	4	2
	N155H	5.0 ± 2.4	3	2
	E92Q + Y143C	**15 ± 10**	5	6
	T97A + Y143C	**>10000**	13	>5000
	G140S + Q148R	**108 ± 54**	7	46
	E92Q + N155H	**25 ± 17**	7	10
	T97A + N155H	**27 ± 13**	3	12
HIV-1	Wild-type	1.5 ± 0.6	14	
	T97A + Y143C	1.5 ± 0.4	4	1
	G140S + Q148R	**6.8 ± 2.7**	4	4
	E92Q + N155H	**3.6 ± 0.7**	4	2

[a]50% effective concentration of dolutegravir (DTG) as measured in the MAGIC-5A single-cycle assay. Values were compiled from the data used to generate Figures 2A and 2D and are expressed as means ± standard deviations. Numbers shown in bold type are significantly greater than the values for the corresponding wild-type strains ($p < 0.05$; ANOVA of $\log_{10}$-transformed EC_{50} values with Tukey's post-test; performed in Prism version 6.0, GraphPad Software, Inc.).
[b]Number of independent determinations for each strain.
[c]Fold change in EC_{50} relative to the corresponding wild-type strain.

[51], a group H HIV-2 isolate with T97A + Y143C was only 5-fold resistant to dolutegravir (this isolate differs from HIV-2$_{ROD9}$ at 24 of 293 amino acid sites in the integrase protein). In addition, the roles of novel INSTI-associated changes (i.e, H51Y, G118R, F121Y, E138A/K, and R263K; [26,34,57-61,63,73]) remain to be determined in HIV-2, and the level of dolutegravir resistance in vitro that correlates with virologic failure in HIV-2–infected patients is unknown. Nonetheless, our findings suggest that, relative to HIV-1, a broader array of amino acid changes in HIV-2 integrase might facilitate cross-resistance between dolutegravir and other INSTI. Phenotypic drug resistance testing of HIV-2 isolates from raltegravir- and elvitegravir-treated patients should be performed as these drugs become more widely available in West Africa, and studies of dolutegravir-based regimens should be conducted in HIV-2–infected individuals, including patients previously treated with other INSTI.

Abbreviations
HIV-1: Human immunodeficiency virus type 1; HIV-2: Human immunodeficiency virus type 2; ARV: antiretroviral; INSTI: Integrase strand transfer inhibitor; EC$_{50}$: 50% effective concentration; DTG: Dolutegravir; RAL: Raltegravir; EVG: Elvitegravir; FDA: Food and drug administration; ANOVA: Analysis of variance.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
RS and GG conceived the study, designed the experiments and prepared the final version of the manuscript. RS performed the experiments, analyzed the data, and drafted the manuscript. DR and CP helped conduct the virologic assays. GG, PS, MS and JM provided intellectual input throughout the study and helped interpret the data. All authors read and approved the final manuscript.

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