The Activity of the Integrase Inhibitor Dolutegravir Against HIV-1 Variants Isolated From Raltegravir-Treated Adults

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Background: Dolutegravir (DTG, S/GSK1349572) is an integrase inhibitor with low nanomolar potency. Susceptibility to dolutegravir and raltegravir was determined for raltegravir-resistant clinical isolates.

Methods: Genotypic and phenotypic susceptibility to integrase inhibitors was examined using 39 clinical isolate samples obtained from 18 adults who had exhibited incomplete viral suppression on a raltegravir-based regimen.

Results: Of 39 samples evaluated, 30 had genotypic and phenotypic resistance to raltegravir. All samples lacking raltegravir resistance retained complete susceptibility to dolutegravir. Of the 30 samples with genotypic evidence of raltegravir resistance, the median level of phenotypic resistance to raltegravir was high (median fold change in inhibitory concentration at 50%, >81; range, 3.7 to >87), while the level of resistance to dolutegravir was close to that of wild-type variants (median fold change, 1.5; range, 0.9–19.0). Longitudinal samples from 5 subjects collected during long-term failure of raltegravir revealed time-dependent general decreases in phenotypic susceptibility to raltegravir, with minimal changes in phenotypic susceptibility to dolutegravir. The median fold change to dolutegravir for isolates containing changes at G140S + Q148H, G140S + Q148R, T97A + Y143R, and N155H (thus including raltegravir signature resistance codons) were 3.75, 13.3, 1.05, and 1.37, respectively.

Conclusions: Dolutegravir retained in vitro activity against clinical isolates obtained from subjects who failed raltegravir-based therapy at near wild-type levels for variants containing the Y143 and N155 resistance mutations. Isolates with Q148 plus additional integrase mutations possessed a broader range of and more reduced susceptibility to dolutegravir.

Key Words: Dolutegravir, DTG, S/GSK1349572, integrase inhibitor, raltegravir resistance, UCSF SCOPE cohort

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INTRODUCTION

With the registration of raltegravir in 2007, the HIV-1 integrase enzyme became the most recent drug target for which an antiretroviral drug has been approved. As with all antiretroviral drug classes, the long-term in vivo utility of integrase inhibitors is limited by the development of drug resistance. The genotypic and phenotypic characteristics of raltegravir resistance have been well described. In clinical studies of raltegravir, subjects with virologic failure and reduced integrase inhibitor susceptibility typically harbor virus with one of 3 signature mutational pathways: Y143, Q148 (typically Q148H in combination with G140S), or N155.[1–4]

Although many characteristics of a drug class can improve as new agents emerge, a key feature for the expanded utility of new drugs against an existing target is a substantially different resistance profile. Dolutegravir (DTG, S/GSK1349572) is a novel integrase inhibitor with a distinct in vitro resistance profile that includes substantial activity against HIV with Y143 or N155H plus secondary raltegravir-associated mutations or against the Q148 mutations alone; a broader activity range is observed for viruses with Q148 pathway genotypes with fold change (FC) resistance generally increasing as the number of secondary mutations increases.[5,6] Among integrase inhibitor–naive subjects, dolutegravir taken once daily has exhibited potent antiretroviral activity in phase 1/2 studies.[7,8] Dolutegravir has demonstrated that a predictable well-characterized exposure-response relationship and low pharmacokinetic variability does not require a pharmacokinetic boosting agent[9] and is currently in phase 3 clinical development. On the basis of extensive experience testing the phenotypic susceptibility of drugs from other antiretroviral drug classes, it is expected that the dolutegravir phenotypic susceptibility of samples from subjects exhibiting virologic failure on raltegravir will predict how well this novel drug will work in raltegravir-experienced subjects. In this report, we describe our initial investigation of the activity of dolutegravir against clinical isolates from subjects experiencing virologic failure while on raltegravir therapy.

METHODS

HIV Isolates

The HIV-1 samples evaluated were from 18 adults and included 8 clinical isolates containing integrase inhibitor resistance mutations from a Monogram Biosciences, Inc (South San Francisco, CA) library set and 31 clinical isolate samples from the University of California, San Francisco Study of the Consequences of Protease Inhibitors Era (SCOPE) cohort.[10] SCOPE is an observational prospective study of HIV-1–infected adults designed to provide a specimen bank of samples with carefully characterized clinical data. Of 39 clinical isolate samples examined, 30 had integrase coding region mutations, and 21 of these were longitudinal samples from 9 subjects. All 8 clinical isolate samples obtained from Monogram Biosciences had evidence of raltegravir resistance, while 22 of the SCOPE samples had evidence of raltegravir resistance. In addition, we studied 11 site-directed mutant control HIV-1 samples (integrase sequences based on NL43).

Viral Genotyping and Phenotyping Assays

Integrase-resistant HIV-1 sample phenotypes were evaluated using the PhenoSense IN assay at Monogram Biosciences, Inc.[2,11,12] Dolutegravir and raltegravir were tested side by side, and inhibitory concentration at 50% (IC_{50}) and $FC\text{-}IC_{50}$ versus wild type were generated. Briefly, 1.6 kb of the HIV-1 pol sequence containing the C-terminal domain of reverse transcriptase, RNase H, and integrase was amplified from subject plasma by reverse transcriptase polymerase chain reaction and transferred into a resistant test vector containing a luciferase reporter gene. Cotransfections of HEK293 cells with integrase-specific resistant test vectors and an amphotropic murine leukemia virus envelope expression vector were performed to produce pseudovirus stocks that contain patient-derived integrase sequences. Virus stocks were used to infect fresh HEK293 cells in the absence or the presence of serial dilutions of the integrase inhibitor test compounds. Susceptibility was calculated by plotting the percent inhibition of virus replication (luciferase activity) versus the $\log_{10}$ drug concentration to derive the IC_{50}. The integrase genotypes of isolates were determined using the GeneSeq IN assay (Monogram Biosciences, San Francisco, CA).

RESULTS

The median IC_{50} for dolutegravir against 9 wild-type isolates was 1.07 nM (range, 0.8–1.6 nM) compared with 9.15 nM for raltegravir (range, 5.2–13.6 nM). Phenotypic resistance to raltegravir and dolutegravir is reported here as the $FC\text{-}IC_{50}$ of the test sample compared with the wild-type sample. The FC for representative raltegravir-resistant clinical isolate virus compared with wild-type HIV-1 is presented in Table 1. Dolutegravir has essentially wild-type levels of activity against the N155H (median $FC\text{-}IC_{50}$, 1.37) and the T97A + Y143R mutants (median $FC\text{-}IC_{50}$, 1.05). Dolutegravir susceptibility is diminished by HIV-1 isolates carrying G140S + Q148H (median $FC\text{-}IC_{50}$, 3.75) and is further diminished by isolates containing G140S + Q148R (median $FC\text{-}IC_{50}$, 13.3). Overall, for the 30 clinical isolate samples with integrase coding region mutations examined, the dolutegravir $FC\text{-}IC_{50}$ ranged from 0.9 to 19.0; the raltegravir $FC\text{-}IC_{50}$ ranged from 3.7 to >87 (a maximum within assay measureable $FC\text{-}IC_{50}$ for raltegravir). As previously reported,[2] resistance to raltegravir is moderate with N155H (median $FC\text{-}IC_{50}$, 19.0) and high with the other representative genotypes (median $FC\text{-}IC_{50}$, >87 for the combinations of G140S + Q148H, G140S + Q148R, and T97A + Y143R).

Longitudinal samples from 5 subjects receiving raltegravir plus optimized background therapy were analyzed for changes in susceptibility to dolutegravir and raltegravir (Fig. 1). Susceptibility to dolutegravir remained at virtually wild-type levels throughout, whereas raltegravir resistance emerged, with some fluctuation, over time.
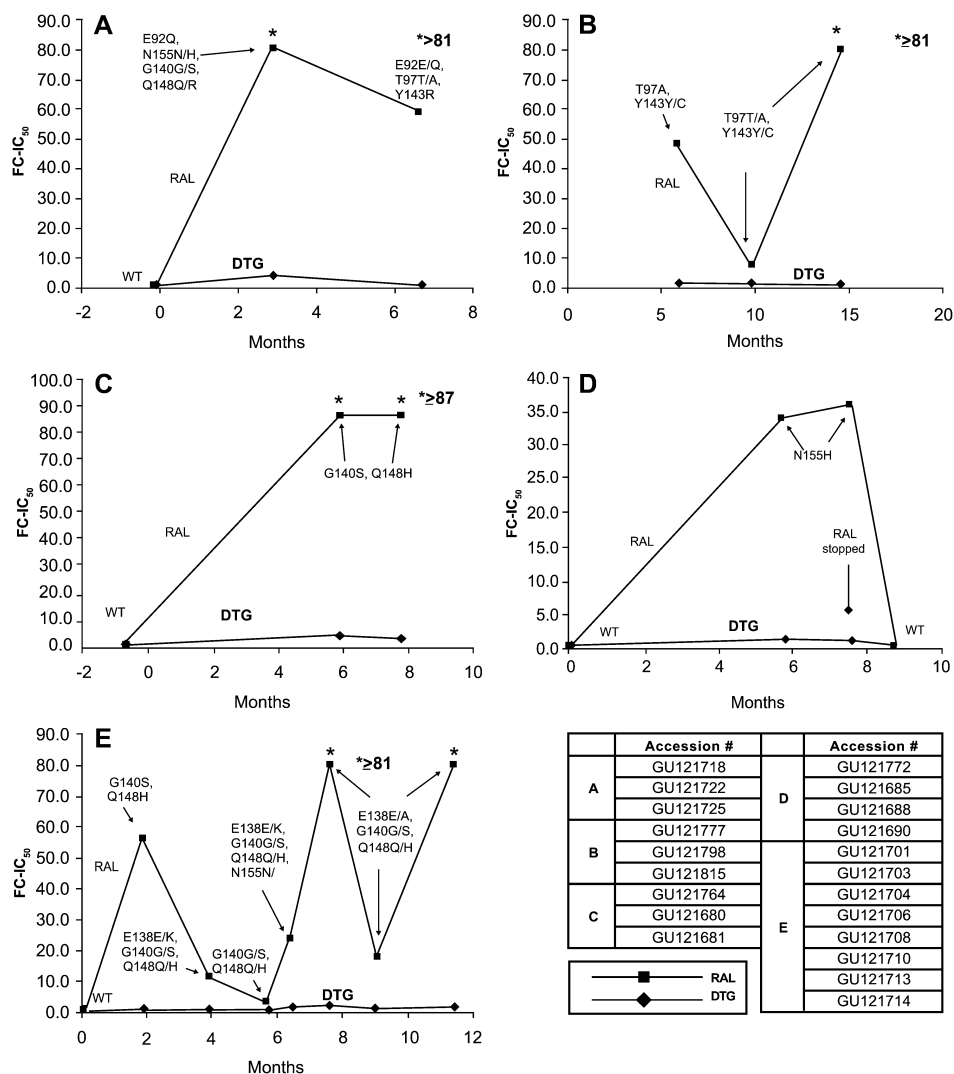
The $FC\text{-}IC_{50}$ of dolutegravir and raltegravir against integrase site-directed mutants and clinical isolates were examined for a more thorough profile of the susceptibility of specific mutant pathways. As shown in Figure 2A, for all single mutants examined, dolutegravir maintained activity to a greater extent than raltegravir ($FC\text{-}IC_{50}$ range for dolutegravir, 0.51–2.45; $FC\text{-}IC_{50}$ range for raltegravir, 1.81–36), with the exception of S153Y; against S153Y, dolutegravir had an $FC\text{-}IC_{50}$ of 2.45 and raltegravir had an $FC\text{-}IC_{50}$ of 1.81. Raltegravir had an $FC\text{-}IC_{50}$ >5 for all single mutants except S153Y. Isolated mutations at the Q148 position (generally considered the most significant pathway for raltegravir resistance) seemed to confer small but measurable decreases in susceptibility to dolutegravir. As shown in Figure 2B, dolutegravir had near wild-type activity against isolates examined with ≥2 mutations and without 148H/K/R (range, 0.87–2.25), whereas raltegravir had an $FC\text{-}IC_{50}$ >5 (range, 3.74 to >81) for all but one (T97T/A + N155N/H; $FC\text{-}IC_{50}$ of 3.74) of this set of mutants. Dolutegravir maintained activity across the isolates examined with combined 140S and 148H/R to a greater extent ($FC\text{-}IC_{50}$ range, 1.38–19.0) than raltegravir ($FC\text{-}IC_{50}$ range, 3.74 to >87; Fig. 2C). Dolutegravir maintained activity to a greater extent across all isolates examined with the grouping of 138K + 148K/R or with ≥3 mutations ($FC\text{-}IC_{50}$ range, 1.58–6.9) than raltegravir ($FC\text{-}IC_{50}$ range, 12 to >81; Fig. 2D).

DISCUSSION

Although raltegravir has exhibited substantial efficacy in clinical trials, it is expected that over time a significant number of individuals in clinical practice will exhibit incomplete viral suppression on this drug and will generate

TABLE 1. Dolutegravir and Raltegravir Phenotypic Susceptibility

Genotype	n	Dolutegravir $FC\text{-}IC_{50}$		Raltegravir $FC\text{-}IC_{50}$	
		Median	Range	Median	Range
N155H	5	1.37	1.22 to 1.45	19.0	14.0 to 36.0
G140S, Q148H	7	3.75	2.05 to 15.0	>87	58.0 to >87
G140S, Q148R	2	13.3	7.57 to 19.0	>87	>87 to >87
T97A, Y143R	2	1.05	1.04 to 1.06	>87	>87 to >87

FIGURE 1. Longitudinal comparison of representative phenotypic susceptibility to dolutegravir and raltegravir from 5 patients (A–E) during the months after the initiation of raltegravir plus optimized background therapy (month 0). GenBank accession numbers are in the embedded table; numbers from top to bottom for each patient (A–E) link to sequences for longitudinal samples from left to right in the corresponding panel. Panels A, B, C, D, and E correspond to UCSF SCOPE isolate numbers 10, 6, 1, 4, and 9, respectively, in Figure 2. Integrase mutations detected are shown. DTG, dolutegravir; FC-IC$_{50}$, fold change in inhibitory concentration at 50%; RAL, raltegravir; WT, wild type. *Represents FC-IC$_{50}$ values >81 or >87 (maximum measurable FC resistance in these assays) as denoted for each chart.

raltegravir-resistant variants. Hence, there is an obvious need for drugs in the integrase inhibitor class that retain activity against isolates containing clinically relevant raltegravir-associated mutations. Elvitegravir (EVG, GS-9137), another integrase inhibitor that is in clinical development, has a resistance profile similar to that of raltegravir.[5,13–16] Preliminary cross-resistance data for the prototype second-generation integrase inhibitor MK-2048, designed to retain activity against HIV with resistance to raltegravir, indicate that it has a distinct resistance profile[17–19]; however, MK-2048 remains in very early clinical development. In vitro data have demonstrated that dolutegravir retains substantial activity against Y143 and N155H pathway virus with additional secondary mutations and against virus with Q148 mutations alone.[5] Dolutegravir activity has a broader range of FC resistance against Q148 pathway virus with additional raltegravir secondary mutations; resistance generally increases with increasing number of mutations.[5,6] Using clinically derived samples, we report that dolutegravir often retains full or near-full activity against variants that possess genotypic and phenotypic resistance to raltegravir. This is particularly true for

isolates containing common raltegravir-associated mutations at positions 143 and 155 in the integrase open-reading frame. Single mutations at Q148 apparently conferred small but measurable susceptibility to dolutegravir. Isolates containing combined mutations at 140 and 148 have less susceptibility to dolutegravir than those isolates with mutations at positions 143 and 155, although in both cases, there is substantially greater susceptibility to dolutegravir than to raltegravir.

From a theoretical perspective, the problem of cross-resistance within the class of strand-transfer–specific integrase inhibitors has been considered a major concern because of the overlapping binding orientation of key pharmacophore elements in the integrase active site of the integrase inhibitors currently in development.[20] However, a close structural comparison of the scaffolds for raltegravir, elvitegravir, and dolutegravir indicates that dolutegravir has a more "streamlined" scaffold.[21,22] A comparison of the position of raltegravir, elvitegravir, and dolutegravir within the catalytic pocket of an HIV-1 model demonstrates that dolutegravir occupies less space between the Mg^{2+} metals at the base of the catalytic pocket and the Y143 at the top of the catalytic loop compared
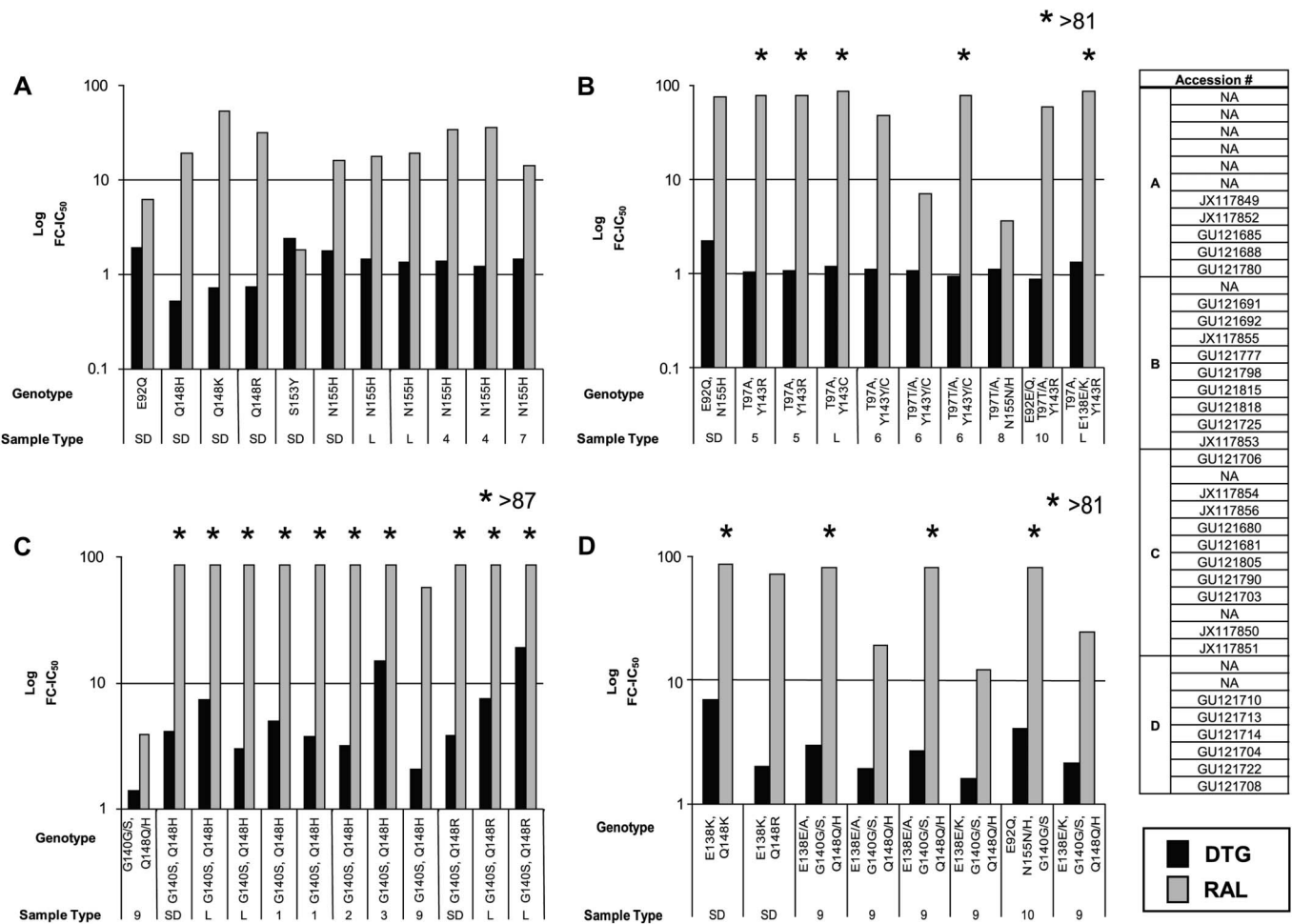
FIGURE 2. Dolutegravir and raltegravir FC-IC$_{50}$ values versus integrase site-directed mutants and clinical isolates from patients who experienced virologic failure with raltegravir. GenBank accession numbers are in the embedded table; NA or accession numbers from top to bottom in each group (A–D) link to sequences from left to right in the corresponding chart. NA indicates that the sample was a site-directed mutant virus. A, Single mutation viruses. B, ≥2 mutation viruses without 148H/K/R. C, 2 mutation viruses with 140S, 148H/R. D, 2 mutation viruses with 138K, 148K/R, or ≥3 mutations. For sample type, SD indicates site-directed mutant, L indicates Monogram Biosciences library isolate, and a single digit indicates a UCSF SCOPE isolate. SCOPE isolate numbers 10, 6, 1, 4, and 9 correspond to panels A, B, C, D, and E, respectively, in Figure 1. DTG, dolutegravir; FC-IC$_{50}$, fold change in inhibitory concentration at 50%; RAL, raltegravir; SCOPE, Study of the Consequences of Protease Inhibitors Era; UCSF, University of California, San Francisco. *Represents FC-IC$_{50}$ values >81 or >87 (maximum measurable FC resistance in these assays) as denoted for each chart.

with both raltegravir and elvitegravir.[21–23] This model renders dolutegravir more independent of the signature mutations that typically impact both of these integrase inhibitors. Overall, the data reported here indicate that although dolutegravir FC resistance is substantially lower than that of raltegravir for all clinical isolates obtained from subjects failing raltegravir-based therapy, there is a decrease in dolutegravir susceptibility for some HIV-1 virus, particularly with mutations at Q148 plus at least one additional raltegravir secondary mutation.

These data, combined with other recent virologic data,[5,6,24] suggest that dolutegravir has a virologic resistance profile distinct from that associated with raltegravir, with some mutation combinations that have a greater decrease in dolutegravir activity. When combined with the potent virologic responses observed during 10-day dolutegravir mono-

therapy given once daily[7] and the good efficacy rates and rapid virologic responses observed in a phase 2b study of antiretroviral-naive subjects,[8] these data suggest that there is a need to continue to study dolutegravir in subjects who exhibit raltegravir resistance and to support the development of dolutegravir for subjects across the treatment spectrum.

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