HIV-1 resistance to dolutegravir: update and new insights

Mark A Wainberg* and Ying-Shan Han

McGill University AIDS Centre, Lady Davis Institute for Medical Research, Jewish General Hospital, Montreal, Quebec, Canada

Abstract

Integrase strand transfer inhibitors (INSTIs) are the latest class of potent anti-HIV drugs. Currently, three INSTIs have been approved by the US Food and Drug Administration: raltegravir (RAL), elvitegravir (EVG) and dolutegravir (DTG). Resistance mutations to RAL and EVG emerge rapidly, and significant cross-resistance between these compounds has been documented. In addition, limited cross-resistance has been observed among DTG, a newer INSTI, and RAL and EVG even though clinical resistance to DTG, or mutations associated with DTG resistance in treatment-naïve patients, has not yet been observed. This review summarises progress in studies on understanding resistance to DTG, mechanisms of possible resistance to DTG, and reasons for the absence of DTG-associated resistance mutations when the drug has been used in first-line therapy.

Keywords: drug resistance, viral fitness, R263K mutation

Introduction

Antiretroviral therapy (ART), which commonly includes at least three different drugs to maximally suppress HIV viral replication, has led to a decrease in HIV-related morbidity and mortality. Currently, 29 anti-HIV drugs in six different classes have been approved by the US Food and Drug Administration (FDA) and are available for HIV therapy. However, HIV can develop resistance to almost all drugs on the basis of mutations that are usually located within the coding regions of the enzymes that serve as drug targets. Integrase strand transfer inhibitors (INSTIs), which block the integration of the HIV viral DNA into host chromosomal DNA, are the latest class of anti-HIV drugs. To date, there are three approved INSTIs: raltegravir (RAL), elvitegravir (EVG) and dolutegravir (DTG). Although both RAL and EVG are highly effective in treatment of HIV-positive patients, both drugs have a low-to-moderate genetic barrier to resistance. Indeed, HIV resistance to RAL and EVG can evolve fairly rapidly both *in vitro* and in patients on the basis of single mutations or combinations of mutations within the HIV integrase [1–4]. Cross-resistance between RAL and EVG has also been observed. However, DTG, a newer INSTI, seems to possess a resistance profile that is different from those of both RAL and EVG. For one thing, DTG often retains activity against RAL- and EVG-resistant viruses and it is the only anti-HIV drug against which HIV has not yet developed resistance mutations in patients who have received treatment with this compound in first-line therapy [5]. This review focuses on the latest findings on resistance mutations to DTG, the underlying mechanisms of possible resistance, as well as reasons for the absence of resistance to DTG and the compounds with which it has been co-administered when these drugs are used in first-line therapy.

Resistance patterns involving DTG

In the case of RAL, primary mutations at positions Y143, Q148 and N155 within the active site of integrase are involved in three major resistance pathways. For EVG, significant primary mutations include T66I, E92Q, N155H and Q148H/K/R. Cross-resistance between RAL and EVG is observed on the basis of mutations at positions 155 and 148. DTG is not often compromised by mutations at N155 but is affected by mutations at position Q148 (Table 1) [6].

*Corresponding author: Mark A. Wainberg, 3999 Chemin de la Côte Ste Catherine, Montréal, QC, H3T 1E2, Canada
Email: mark.wainberg@mcgill.ca

Several mutations that are potentially involved in resistance to DTG have been identified either in culture or in the clinic, and these substitutions have occurred at positions F121, S153, G118, E138 and R263 [7,8]. These mutations, alone or in association with secondary mutations, can influence susceptibility to DTG and/or impair viral replicative fitness to varying extents (Table 2). It has been shown, for example, that the R263K mutation in integrase confers low-level resistance to DTG (fold change, FC=2.3-fold) [8]. However, this mutation also impairs integrase strand transfer activity and diminishes viral replication capacity. M50I was identified as an accessory mutation in association with R263K and was selected under pressure with DTG. Usually, secondary mutations, in combination with primary mutations, increase drug resistance while also restoring viral replication fitness. The natural polymorphism M50I alone does not impair either strand transfer activity or viral replication capacity. Unusually, the addition of M50I to R263K increases resistance to DTG by ~15-fold but it does not restore viral infectivity and replication capacity [9]. A combination of H51Y with R263K increases resistance to DTG by roughly 10-fold, but it also dramatically decreases viral replication capacity by approximately 90%, and is accompanied by a near 80% decrease in enzyme strand transfer activity [3]. Recent studies have shown that the addition of E138K to R263K, while modestly increasing resistance to DTG in cell culture (FC=4.3), slightly increased susceptibility to DTG in cell-free strand-transfer assays from FC≈3 to FC≈4.4. The combination of E138K and R263K decreased integrase strand transfer activity to about 60% of that obtained with a wild-type (WT) enzyme and also failed to restore viral infectivity (~two-fold decrease) or replication capacity [10].

Mutations in integrase at positions R263K, G118R, H51Y and E138K have been characterised as conferring low-level resistance to DTG. A recent study tested the ability of DTG-resistant viruses harbouring either R263K or G118R together with H51Y to develop further resistance against reverse transcriptase inhibitors such as lamivudine or nevirapine in tissue culture selections. In the presence of lamivudine, WT viruses developed the M184V/I mutation resistance to lamivudine in as little as 6 weeks. The H51Y mutation alone had little or no effect on the speed with which M184V/I occurred, but viruses harbouring R263K were delayed in regard to the appearance of M184V by several weeks. Similarly, the V106A mutation that confers resistance to nevirapine was detected after 6 weeks in the case of WT virus but only appeared between weeks 11 and 14 in selections performed with viruses carrying R263K. G118R- and H51Y/G118R-containing viruses did not develop relevant resistance mutations

to lamivudine or nevirapine over longer than 25 weeks. These results clearly show that the R263K or G118R mutations, alone or in combination with H51Y, can delay the emergence of mutations responsible for resistance to both nevirapine and lamivudine. It may be that these delays have been caused by the decreased viral replication capacity associated with DTG resistance mutations [11], since no compensatory mutation has been identified for DTG in tissue culture selection experiments that have continued for more than 4 years [5,12]. Interestingly, combinations of R263K with primary resistance mutations for RAL and EVG at positions 143, 148 and 155 have resulted in vastly diminished enzymatic activity that may be incompatible with viral survival, which helps to explain why R263K has never been observed in the clinic together with primary RAL or EVG mutations [13].

As stated above, no virological failure in the context of development of resistance to DTG in treatment-naïve individuals has been reported [5]. This may be partially explained by the fact that the presence of mutations that confer resistance to DTG can impair the ability of HIV to develop further resistance against other drugs such as lamivudine and nevirapine. Thus, DTG-containing regimens may not lead to virological failure if the R263K mutation emerges. This hypothesis will eventually be verified by ultrasensitive sequencing of the integrase gene in residual plasma viral RNA and from the DNA of lymphocytes of patients who have been successfully treated with DTG [11].

Since no treatment-naïve patient treated with DTG has yet developed resistance to DTG, and since the R263K mutation confers low-level resistance to DTG in tissue culture, it may be expected that DTG should play a role in limiting HIV persistence. To study the impact of the R263K mutation on HIV replication capacity and the ability of HIV to establish or be reactivated from latency and/or spread through cell-to-cell transmission, a series of constructs containing green fluorescence protein (GFP) was created by site-directed mutagenesis (i.e. pNL4-3-IRES-EGFP-INR263K, pNL4-3-IRES-EGFP-INE138K and pNL4-3-IRES-EGFP-INE138K/R263K). The relevant replication-competent reporter viruses were produced and used to study the effects of the R263K mutation on HIV replication capacity and the ability to establish latency and/or spread through cell-to-cell transmission in Jurkat cells. The results showed that the R263K substitution did indeed result in diminished replication capacity and infection. However, the DTG-resistant viruses could still be efficiently transmitted via cell-to-cell contact, and were as likely to establish and be reactivated from latent infection as WT virus [14].

Obviously, baseline sequences in integrase might affect any given mutation in terms of susceptibility to DTG. Indeed, DTG can remain effective against RAL-resistant variants that contain mutations at positions N155H, Y143C, N155H/Y143C and G140S/Q148H in different cells, including C8166, human primary monocyte-derived macrophages (MDMs), and peripheral blood mononuclear cells (PBMCs) [15]. Similarly, it has been shown that DTG is effective against patient-derived RAL-resistant variants that contain either Y143 or N155 mutations in both macrophages and CD4+ T cells, with the exception of Q148H/R-bearing variants that display reduced susceptibility (FC=5.5–19) [16]. In addition, a RAL-treatment-experienced patient harbouring the N155H mutation resistant to RAL, was switched to a DTG-containing regimen for about 10 months. Subsequently, mutations at positions T97A and E138K in integrase developed and displayed 37-fold resistance to DTG. After use of DTG for 10 more months, the sequential acquisition of mutations at A49P, L68FL and L234V led to further resistance to DTG (FC=63-fold). Of the foregoing substitutions, A49P and L234V are novel. It was further observed that the serial acquisition of DTG-resistance mutations was associated with deficits in viral replicative capacity (≈41%) relative to levels observed (101%–187%) prior to the use of DTG [17].

The G118R mutation was also detected in a patient harbouring a subtype CRF02_AG virus and for whom treatment with RAL was failing. In addition, an F121Y mutation was detected alongside other mutations in another patient harbouring subtype B and for whom RAL treatment was failing. Phenotypic susceptibility analyses in cell culture showed that the G118R and F121Y mutations conferred broad cross-resistance to all three currently

Table 1. Resistance pathways for each of RAL, EVG and DTG

	Mutational pathways	Fold resistance		
		RAL	EVG	DTG
Y143 pathway	Y143C	<10	<2	<2
	Y143R	<50	<2	<2
	T97A/Y143C	>100	<2	<2
	T97A/Y143R	>100	<2	<2
	L74M/T97A/Y143G	<50	ND	<2
	L74M/T97A/E138A/Y143C	<20	ND	<2
N155 pathway	N155H	<50	<50	<2
	E92Q/N155H	<100	>100	<10
	L74M/N155H	<50	<50	<2
Q148 pathway	Q148H	<20	<10	<2
	Q148K	<100	<100	<2
	Q148R	<50	<100	<2
	E138K/Q148H	<10	<20	<2
	E138K/Q148K	>100	>100	<20
	E138K/Q148R	>100	>100	<10
	G140S/Q148H	>100	>100	<20
	G140S/Q148K	<10	<100	<2
	G140S/Q148R	>100	>100	<10
	E138A/G140S/Y143H/Q148H	>100	ND	<50
R263K pathway	R263K	<1	3	4
	R263K/H51Y	3–5	3	4–6

Table 2. Effects of mutations in integrase on resistance to INSTIs, viral replication capacity and strand transfer activity

Genotype	Virus	Cell type	Susceptibility to INST (Fold change)			RC (Fold change)	STA (Fold change)	Ref
			RAL	EVG	DTG			
WT	NL43	TZM-bl cells	1	1	1	1	1	[9]
M501			0.47	5.45	1.94	0.92	1.1	
R263K			1.85	21.4	8.55	0.7	0.22	
M501/R263K			3.56	34.44	15.59	0.7	0.31	
WT	NL43	PhenoSense	1	1	1	1	1	[3]
H51Y			1.11	2.06	1.25	0.89	1.07	
R263K			1.21	3.28	1.95	0.7	0.45	
H51Y/R263K			2.94	41.5	6.95	0.11	0.2	
WT	NL43	TZM-bl cells	1	1	1	1	1	[10]
E138K			1	0.8	0.4	0.83	2.4	
R263K			1.2	21.8	2.3	0.72	0.5	
E138K/R263K			1	16	4.3	0.71	0.6	
WT	NL43	PM1 cells	1	1	1	1	1	[20,23]
G118R			0.78	1	—	0.02	0.09	
G118R/H51Y			—	—	—	—	0.23	
G118R/E138K			2.33	2	—	0.13	0.44	

RC: replication capacity; STA: strand transfer activity; WT: wild-type

used INSTIs. In fact, higher levels of resistance were reported for clinical isolates than were observed for site-directed mutants containing the same substitutions in an NL43 backbone (Table 2) [18]. These results suggest that DTG should be used with caution in INSTI salvage therapy for patients for whom RAL-containing regimens have failed and/or that such patients may require that the DTG be protected by other active drugs in this setting.

Mechanisms of HIV resistance to DTG

Considerable progress has been made on mechanisms of HIV resistance to DTG and other INSTIs. Generally, it is accepted that DTG is effective against many RAL- and EVG-resistant variants because binding of DTG to integrase–DNA complexes is much longer than that of either RAL or EVG [4,12,16,19]. Biochemical studies have shown that the R263K mutation in integrase, which confers low-level resistance to DTG, results in decreases in 3′-processing and strand transfer activities. Homology modelling of the intasome and strand transfer complex from wild-type and R263K-containing integrases revealed altered interactions in regard to integrase–DNA binding and an integrase–DNA binding assay showed that the R263K mutation decreases integrase–viral DNA binding [8]. Similarly, biochemical studies have shown that the G118R mutation in integrase greatly decreases its strand transfer activity but does not affect 3′-processing activity [20]. Furthermore, low levels of resistance to DTG that are conferred by mutations in integrase correlate with decreases in strand transfer activity and viral replication capacity (Table 2).

A recent study examined the homology modelling of a constructed tetrameric HIV-1 intasome to reveal the molecular mechanism of the cross-resistance mutations E138K/Q148K to each of RAL, EVG and DTG. Molecular dynamics simulations and residue interaction network analysis revealed that P145 in the 140S loop (G140–G149) of the intasome can have strong hydrophobic interactions with INSTIs and it is involved in conformation rearrangement at the active site of the HIV-1 intasome. A systematic residue interaction network analysis demonstrated that communications between residues in resistant mutants are increased compared with those of the HIV-1 intasome. In addition, the chelating ability of the oxygen atoms in INSTIs (e.g. RAL and EVG) to $Mg2^{+}$ at the active site of resistance mutations was reduced due to conformational change; this is most probably responsible for cross-resistance [21]. More recently, a computational analysis of the G118R and F121Y mutations, conferring high resistance to RAL, EVG and DTG, showed that the G118R and F121Y mutations in integrase were associated with reduced binding affinities to each of the INSTIs studied, due to a decreased number of hydrogen bonds compared with WT complexes [18]. These findings provide valuable information on the mechanisms of resistance to INSTIs and will hopefully be useful for the structure-based design of novel INSTIs with better cross-resistance profiles.

Perspectives

Patients receiving DTG-containing regimens in first-line therapy have achieved high rates of success with up to 90% of individuals showing a drop in viral load to below 50 copies of viral RNA per mL. Even though therapy has failed for some patients, no resistance mutations to either DTG or to the nucleoside and nucleotide drugs with which it has been co-administered have been identified in patients who had been treatment naïve. Moreover, the selection of resistance to DTG in cell culture has yielded only two mutations that confer low-level resistance, but in association with decreased viral replication capacity. Furthermore, no secondary compensatory mutations that might augment resistance and restore viral replication capacity have been observed in tissue culture selection experiments over longer than 4 years. These results may be explained by the fact that the viruses containing DTG-resistant mutations are relatively replication impaired and may be unable to replicate efficiently in patients. Therefore, we have speculated that the development of low-level resistance to DTG in first-line therapy might not have adverse clinical consequences and that DTG might be a useful agent to employ in treatment as prevention (TasP) protocols, since its use might obviate the problem of drug resistance while reducing viral load on a population level; such a strategy could eventually result in vastly diminished rates of HIV

transmission [3,12].

This hypothesis could be tested in a study in which DTG is employed as a monotherapy in treatment-naïve patients. In such a situation, resistance mutations to DTG in both the RNA of patient plasma samples as well as in the DNA of patient lymphocytes would need to be intensively monitored by ultrasensitive sequencing methods. Our hypothesis would be partially validated if the results showed an absence of resistance mutations to DTG, similar to the findings of the phase III clinical trials on the use of DTG triple therapy to treat first-line HIV-infected subjects. Moreover, a number of cycles of DTG monotherapy might help to convert HIV in latent reservoirs to impaired forms in the aftermath of activation of such reservoirs, if compensatory mutations were unable to develop. This concept could be tested in animal models such as rhesus macaques that are infected by simian immunodeficiency virus (SIV) or in humanised mice that are infected by HIV [12]. Of course, the fact that viral evolution may be significantly impaired in the case of R263K/H51Y-containing viruses, as shown in the studies on resistance to lamivudine and nevirapine cited above, may also mean that changes in relevant viral antigens may also be much less likely to occur. In this context, anti-HIV immune responsiveness may remain durable over very long periods of time and also limit the likelihood of viral rebound.

Further characterisation of the resistance profile of DTG in both tissue culture and the clinic is essential. More sensitive assays such as next-generation sequencing for the detection of low-level viraemia and minority resistant variants will be important. The development of new classes of anti-HIV drugs with high genetic barriers for resistance that do not show cross-resistance with current drug classes is still needed [22].

References

1. Quashie PK, Mesplede T, Wainberg MA. Evolution of HIV integrase resistance mutations. *Curr Opin Infect Dis* 2013; **26**: 43–49.
2. Grobler JA, Hazuda DJ. Resistance to HIV integrase strand transfer inhibitors: *in vitro* findings and clinical consequences. *Curr Opin Virol* 2014; **8c**: 98–103.
3. Mesplede T, Quashie PK, Osman N *et al*. Viral fitness cost prevents HIV-1 from evading dolutegravir drug pressure. *Retrovirology* 2013; **10**: 22.
4. Geretti AM, Armenia D, Ceccherini-Silberstein F. Emerging patterns and implications of HIV-1 integrase inhibitor resistance. *Curr Opin Infect Dis* 2012; **25**: 677–686.
5. Mesplede T, Wainberg MA. Is resistance to dolutegravir possible when this drug is used in first-line therapy? *Viruses* 2014; **6**: 3377–3385.
6. Abram ME, Hluhanich RM, Goodman DD *et al*. Impact of primary elvitegravir resistance-associated mutations in HIV-1 integrase on drug susceptibility and viral replication fitness. *Antimicrob Agents Chemother* 2013; **57**: 2654–2663.
7. Kobayashi M, Yoshinaga T, Seki T *et al*. *In vitro* antiretroviral properties of S/GSK1349572, a next-generation HIV integrase inhibitor. *Antimicrob Agents Chemother* 2011; **55**: 813–821.
8. Quashie PK, Mesplede T, Han YS *et al*. Characterization of the R263K mutation in HIV-1 integrase that confers low-level resistance to the second-generation integrase strand transfer inhibitor dolutegravir. *J Virol* 2012; **86**: 2696–2705.
9. Wares M, Mesplede T, Quashie PK *et al*. The M50I polymorphic substitution in association with the R263K mutation in HIV-1 subtype B integrase increases drug resistance but does not restore viral replicative fitness. *Retrovirology* 2014; **11**: 7.
10. Mesplede T, Osman N, Wares M *et al*. Addition of E138K to R263K in HIV integrase increases resistance to dolutegravir, but fails to restore activity of the HIV integrase enzyme and viral replication capacity. *J Antimicrob Chemother* 2014; **69**: 2733–2740.
11. Oliveira M, Mesplede T, Quashie PK *et al*. Resistance mutations against dolutegravir in HIV integrase impair the emergence of resistance against reverse transcriptase inhibitors. *AIDS* 2014; **28**: 813–819.
12. Wainberg MA, Mesplede T, Raffi F. What if HIV were unable to develop resistance against a new therapeutic agent? *BMC Med* 2013; **11**: 249.
13. Anstett K, Mesplede T, Quashie P *et al*. Primary mutations that confer resistance to raltegravir and elvitegravir are incompatible with the R263K mutation that is associated with low-level resistance to dolutegravir. *20th International AIDS Conference*. July 2014. Melbourne, Australia. [MOPDA0105].
14. Bastarache SM, Mesplede T, Donahue DA *et al*. Fitness Impaired Drug Resistant HIV-1 Is Not Compromised in Cell-to-Cell Transmission or Establishment of and Reactivation from Latency. *Viruses* 2014; **6**: 3487–3499.
15. Pollicita M, Surdo M, Di Santo F *et al*. Comparative replication capacity of raltegravir-resistant strains and antiviral activity of the new-generation integrase inhibitor dolutegravir in human primary macrophages and lymphocytes. *J Antimicrob Chemother* 2014; **69**: 2412–2419.
16. Canducci F, Ceresola ER, Saita D *et al*. *In vitro* phenotypes to elvitegravir and dolutegravir in primary macrophages and lymphocytes of clonal recombinant viral variants selected in patients failing raltegravir. *J Antimicrob Chemother* 2013; **68**: 2525–2532.
17. Hardy I, Brenner B, Quashie P *et al*. Evolution of a novel pathway leading to dolutegravir resistance in a patient harbouring N155H and multiclass drug resistance. *J Antimicrob Chemother* 2014.
18. Malet I, Gimferrer Arriaga L, Artese A *et al*. New raltegravir resistance pathways induce broad cross-resistance to all currently used integrase inhibitors. *J Antimicrob Chemother* 2014; **69**: 2118–2122.
19. Hightower KE, Wang R, Deanda F *et al*. Dolutegravir (S/GSK1349572) exhibits significantly slower dissociation than raltegravir and elvitegravir from wild-type and integrase inhibitor-resistant HIV-1 integrase-DNA complexes. *Antimicrob Agents Chemother* 2011; **55**: 4552–4559.
20. Quashie PK, Mesplede T, Han YS *et al*. Biochemical analysis of the role of G118R-linked dolutegravir drug resistance substitutions in HIV-1 integrase. *Antimicrob Agents Chemother* 2013; **57**: 6223–6235.
21. Xue W, Jin X, Ning L *et al*. Exploring the molecular mechanism of cross-resistance to HIV-1 integrase strand transfer inhibitors by molecular dynamics simulation and residue interaction network analysis. *J Chem Inf Model* 2013; **53**: 210–222.
22. White KL, Raffi F, Miller MD. Resistance analyses of integrase strand transfer inhibitors within phase 3 clinical trials of treatment-naive patients. *Viruses* 2014; **6**: 2858–2879.
23. Bar-Magen T, Sloan RD, Donahue DA *et al*. Identification of novel mutations responsible for resistance to MK-2048, a second-generation HIV-1 integrase inhibitor. *J Virol* 2010; **84**: 9210–9216.