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Is Drug Resistance in First-Line Therapy against All Antiretroviral Agents Inevitable?

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Abstract

Drug resistance has been observed in regard to all anti-retroviral drugs that have been studied until now. The most recent drug to be approved for use in HIV therapy is dolutegravir, an HIV integrase inhibitor, that was approved for therapy by the Food and Drug Administration in the United States in 2013. Dolutegravir is the only HIV drug that has not selected for resistance mutations in the clinic when used as part of first-line therapy. We have hypothesized that this is due to the long binding time of dolutegravir to the integrase enzyme as well as greatly diminished replication capacity on the part of viruses that might become resistant to dolutegravir and that are unable to successfully replicate in infected individuals.

Keywords: HIV integrase; Dolutegravir; Resistance; R263K; Viral fitness; Eradication

Introduction

The use of three antiretroviral (ARV) drugs in combination to treat HIV-infected individuals is common, and simplified regimens may now often include three different agents that are co-formulated within a single tablet. A number of reasons account for the fact that the use of triple ARV therapy since 1996 has now led to rates of therapeutic success that have increased to over 90%, based on suppression of plasma viremia to below 50 copies of viral RNA/ml. First, adherence to ARV regimens is now far easier than previously, due to the fact that dosing regimens have become simplified, often because of the use of co-formulations, which may only need to be taken once-daily. Second, ARV regimens have become far less toxic and more tolerable over time and this has also promoted adherence as well as diminished the likelihood of development of HIV drug resistance against individual drugs [1,2]. Finally, the drugs that are now used in therapy are far more potent than those that were in use only 10 years ago and are often members of new drug classes that did not previously exist.

This notwithstanding, the use of ARVs in first-line regimens has always been associated with some degree of treatment failure and drug resistance. Indeed, scientists have mapped out a wide array of drug resistance mutations that are located within each of the protease, reverse transcriptase, and integrase enzymes of HIV-1 that are the targets of HIV therapy, and have documented how each of these mutations may lead to diminished likelihood of a favorable clinical response to each drug and have documented the mechanisms that underlie such drug resistance [1]. Of course, the phase III clinical trials that led to the approval of each of the ARVs now used for therapy also provided valuable information on the types of mutations that were most likely to be identified following levels >50 copies RNA/ml in plasma. Resistance to the first integrase strand transfer inhibitors (INSTIs) i.e. raltegravir (RAL) and elvitegravir (EVG), was also demonstrated both in clinical trials and in tissue culture drug selection studies [3-7].

However, the novel INSTI termed dolutegravir (DTG) has now yielded the most robust results ever obtained in HIV phase III clinical trials [8]. Although, approximately 88% of patients who received DTG in these studies attained suppression of viral load to <50 copies RNA/ml, what is remarkable is that none of the participants developed a single drug resistance-related mutation that was associated with either DTG or the nucleoside drugs that were used together with DTG as a part of triple combination therapy. The 10-15% of patients in the trials who did not respond to therapy possessed detectable levels of viral load in plasma, perhaps for reasons of non-adherence [9,10], but did not harbor any detectable drug-resistance mutations. Although this situation is somewhat similar to the rarity with which resistance against boosted protease inhibitors (PIs) has been detected after virological failure (VF), it is also true this has been primarily investigated for mutations in the viral protease (PR) gene [1] and not at gag cleavage sites. In addition, the M184V mutation, associated with resistance to 3TC, was present in some cases of failure involving boosted PIs but has not been detected in any cases of viral rebound following DTG use in first-line therapy.

Does Lack of Fitness Prevent Potentially Dtg-Resistant Hiv-1 from Growing in Patients?

A hypothesis that has been advanced to explain these findings is that viruses that become resistant to DTG may be so replication-incapacitated as to not be able to efficiently grow; thus, such variants might not be detectable in patient plasma [11]. For example, it is known that DTG selects a mutation at position R263K in the integrase gene in tissue culture and in some treatment-experienced patients and that this mutation diminishes the enzymatic activity of the integrase enzyme as well as viral replication capacity [12]. Indeed, similar results to the above have been obtained with the two other approved integrase inhibitors RAL and EVG [11]. However, in those cases, the presence of an initial mutation was often quickly followed by the appearance of a

second substitution that had the dual effect of significantly increasing the level of drug resistance to an extent that might preclude the further clinical benefit of the drug in question while simultaneously increasing viral replication capacity or viral fitness to close to wild-type levels (Table 1). It is key, by way of contrast, that the selection of a second DTG mutation only marginally increased levels of DTG resistance while simultaneously causing viral replication capacity to diminish by as much as 80% compared to wild-type. This change was further corrected with a further diminution in the activity of the HIV integrase enzyme in both 3' synthetase assays and integrase strand transfer assays. Furthermore, this also resulted in a dramatic reduction in the ability of the integrase enzyme to incorporate newly synthesized viral DNA into the DNA of infected host cells [11,12].

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

An additional consequence of diminished viral replication capacity, and, by inference, diminished ability to further evolve and mutate, may be that the anti-HIV immune responsiveness remains durable and active against HIV and HIV-infected cells over far longer periods of time that would otherwise be the case. This is a concept that could be tested experimentally by showing, as an example, that levels of autologous neutralizing antibodies against a given isolate remain elevated over months or years rather than for only weeks, as has been shown in a number of studies. A second example is the recent demonstration that HIV that contains both the R263K and H51Y mutation in integrase seems to be unable to develop the M184V reverse transcriptase resistance mutation that was quickly generated under 3TC pressure by HIV that was either wild-type or that contained either the R263K or H51Y mutations alone.

Of course, secondary and/or tertiary drug resistance mutations often play a compensatory role in regard to replication for many microorganisms besides HIV, including viruses that display resistance against specific antiviral drugs and bacteria that are resistant to numerous antibiotics. In the case of HIV, compensatory mutations that simultaneously increase viral replication while augmenting overall levels of drug resistance have been documented for members of each of nucleoside reverse transcriptase inhibitor (NRTI), non-nucleoside RT inhibitor (NNRTI), and protease inhibitor (PI) families of drugs [1]. The fact that no such mutation has been identified for DTG, represents a unique situation that is bolstered by the results of tissue culture selection experiments that have yielded only two distinct mutations that greatly diminish viral replicative capacity but never a third compensatory mutation over more than four years of tissue culture selection studies [11].

Could Integrase Inhibitors Assist in HIV Eradication Strategies?

What might transpire if viruses that are resistant to DTG cannot be compensated by additional mutations within integrase and if such viruses are truly at a severe replication disadvantage in comparison with wild-type HIV or with viruses that are not as compromised in replication capacity?. What if it turned out that DTG can retain clinically significant antiviral activity, despite the presence of one or two resistance mutations that are associated with this compound? The fact that the level of resistance conferred against DTG by the combination of two such mutations within integrase is <6-fold and that the ability of DTG to bind to the integrase enzyme and remain associated with it is very long, i.e. >60 hours, suggests the plausibility of this hypothesis. In addition, the R263K mutation only diminished this level of binding by about 50% ([13], unpublished data) and this is still longer than the dissociation half-life of EVG and RAL for the wild-type enzyme; this suggests that the development of low-level resistance against DTG in first-line therapy might not have adverse consequences either virologically or clinically.

DTG was only approved for treatment in the USA approximately 18 months ago and all of the clinical data that pertain to this compound have been obtained as part of randomized clinical trials. This means that further support for our hypothesis may only accrue after DTG has been widely prescribed, including under conditions in which considerable non-adherence to treatment can be expected outside of clinical trial settings. The data now suggest that patients who may become resistant to DTG will still respond to RAL, but further clinical experience will be needed to substantiate this point as well as to

provide information on the concept of the sequential use of different integrase inhibitors in the clinic.

How could this hypothesis be tested? First, a study could be contemplated in which DTG is employed as monotherapy in treatment-naive subjects, even though proof-of-concept results should first be obtained in relevant animal models. In addition, it would be salutary to conduct studies in which DTG together with 3TC are first studied as a two drug regimen in comparison with DTG plus two NRTIs and to show equivalence between the two arms of such a study before proceeding. If the results obtained are similar to those observed in the phase III clinical trials, a partial validation of the hypothesis to explain the absence of resistance in the phase III trials will have been obtained. It goes without saying that such studies would need to be accompanied by intense virologic monitoring for resistance mutations that should include the use of ultrasensitive sequencing for identification of DTG resistance mutations in the DNA of patient peripheral blood mononuclear cells as well as in the RNA of patient plasma samples.

However, it should be noted that some clinical validation of the significance of the R263K mutation has already been obtained in the SAILING-clinical trial that compared the use of RAL against DTG in treatment-experienced patients who had undergone previous failures of their therapeutic regimens but who had never before been treated with an integrase inhibitor [14]. All of the patients in this study at baseline possessed drug resistance mutations that might have compromised the antiviral activity of multiple ARVs in the regimens that they received, but they did not possess integrase mutations because they had not received any INSTIs to that point. The results showed that DTG was superior to RAL at suppression of viral load in these and that the most common drug resistance mutation to have appeared, in only two patients, in the DTG arm of the study was R263K. In contrast, failure on the RAL arm of the study led to a broad array of RAL-associated mutations in integrase. Although, the patients who received DTG and who possessed the R263K mutation have apparently continued to be clinically well, new information is needed in regard to mutations that may have developed over time in such individuals. The data to date suggest that subsequent viral evolution did not take place [14]. However, important questions of durability of responsiveness remain unanswered.

What about Treatment Failures on DTG.

How can we explain the fact that the non-adherent patients who received DTG in first-line therapy did not generate any resistance mutations to any of the drugs that they received as a part of their therapy. One answer, of course, is that DTG may have the highest barrier to resistance of all anti-HIV compounds, a notion that is consistent with the hypothesis outlined in this paper. We are now trying to access clinical specimens from circulating lymphocytes and from the lymphocytes that are present in gut tissue and other body compartments of patients receiving DTG in first-line therapy to try to shed light on this topic. Of course, the presence of defective viral forms that contain integrase resistance mutations that relate to the R263K pathway might be more common than thought until now and defective viruses might not easily be able to replicate; although this may complicate matters, the results should be available within 6 months.

Dolutegravir and Other Integrase Inhibitors for the Management of HIV-Positive Individuals

DTG is an agent to be considered for use in first-line therapy, since the development of R263K and a subsequent resistance mutation may not confer any deleterious effect in regard to viral replication. However, and by way of contrast, the prior development of mutations associated with resistance against RAL or EVG may compromise the use of DTG in salvage therapy. Indeed, the Viking studies showed that DTG can only be used in about 60% of cases to salvage patients who were first treated with RAL or EVG and who failed those regimens with resistance-associated mutations [15]. Furthermore, the durability of DTG use in this setting remains a question of relevance. Although some patients who first failed RAL- or EVG-based regimens did respond virologically when treated with DTG as part of second-line therapy, many such patients who first failed RAL and/or EVG will have exhausted many treatment options For these reasons, treatment should be initiated with the best drugs that are approved for therapy, and the concept of sequential integrase inhibitor usage may be illusory. Of course, it is also true that relatively few first-line patients have ever failed RAL or EVG-based regimens. In addition, none of a series of secondary mutations to R263K at positions H51Y, M50L, or E138K has ever been shown to restore viral replication capacity, although these may add somewhat to the levels of resistance to DTG associated with the R263K mutation [16-18].

Summary

This article makes reference to concepts that should first be studied in animal models such as humanized mice that are infected by HIV or rhesus macaques that are infected by simian immunodeficiency virus (SIV). Although some clinicians have experimented with monotherapy in the past and are likely to do so again, it is likely that further justification for such studies may first come from clinical trials in which patients are first suppressed with DTG plus two other drugs and then maintained on DTG monotherapy.

Some might argue that the development of compensatory mutations associated with DTG might only be a matter of time. However, each passing day without resistance to DTG in first-line therapy lends credence to the hypothesis outlined here. It should also be noted that failure to develop resistance to DTG or to experience a rebound in viral load in DTG-treated patients could conceivably lead to an inability of people treated with DTG to transmit HIV [19,20]. Hence, the development of DTG could turn out to have profound implications for future HIV transmission, the sustainability of the HIV epidemic, and, by corollary, for public health. Such a positive consequence might require that all future HIV-infected persons worldwide be initiated on DTG as a part of a first-line therapeutic regimen.

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Authors' Contributions

Each of Mark A Wainberg and Thibault Mesplède conceived the idea for this article and contributed to the writing. All authors read and approved the final manuscript.

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