Resistance to inhibitors of the human immunodeficiency virus type 1 integration

ABSTRACT

This review will summarize the role of integrase in HIV-1 infection, the mechanism of integrase inhibitors and resistance with an emphasis on raltegravir (RAL), the first integrase inhibitor licensed to treat HIV-1 infection.

Keywords: antiretrovirals, integrase inhibitors, raltegravir.

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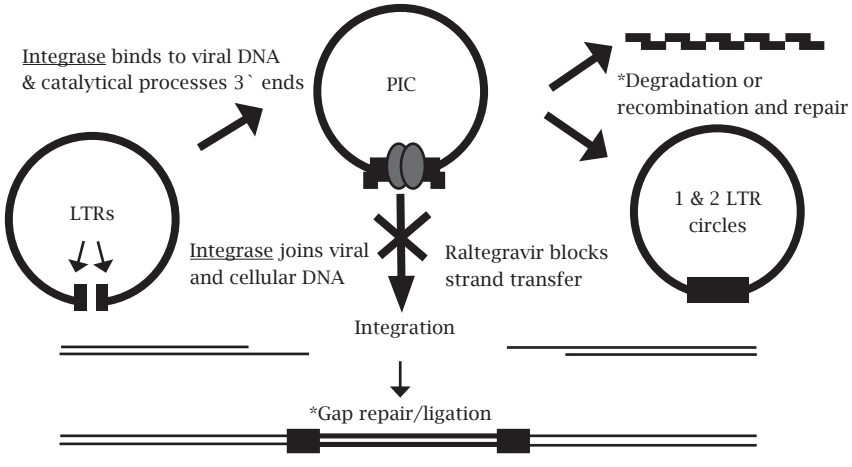
INTRODUCTION

The replication of all retroviruses including the Human Immunodeficiency Virus Type 1 (HIV-1) requires three viral enzymes: reverse transcriptase (RT), protease (Pr) and integrase (In). The development of inhibitors of reverse transcriptase and protease and the subsequent introduction of combination drug regimens which enhance the overall efficacy and durability of therapy revolutionized the treatment of HIV-1 infection in the mid 1990's. As the last of the three essential HIV-1 enzymes, integrase was considered an equally attractive target for antiretroviral drug development as protease and reverse transcriptase, but it is only a decade later that the first integrase inhibitor, raltegravir (RAL, MK-0518) achieved regulatory approval, reviewed in Cahn,[1] while other integrase inhibitors including elvitegravir (EVG, GS-9137, JTK303) and soltegravir (S1360) are still in clinical development. In this review we will provide an overview of the biology and biochemistry of integrase inhibitors and an update on our current understanding of resistance to this newest class of antiretroviral agents.

The role of integrase in HIV-1 replication

Integrase mediates the irreversible insertion or integration of the HIV-1 DNA into the host genomic DNA.[2-4] Integration is required to maintain the HIV-1 genome in the infected cell and for the efficient expression of all viral proteins leading to the generation of new viruses. Integrase mediates three highly specific and coordinated steps which are required for integration (Figure 1). Integrase initially *assembles* at specific sequences within the long terminal repeat

Figure 1: Schematic representation of the multi-staged process of integration.



Note: *cellular functions

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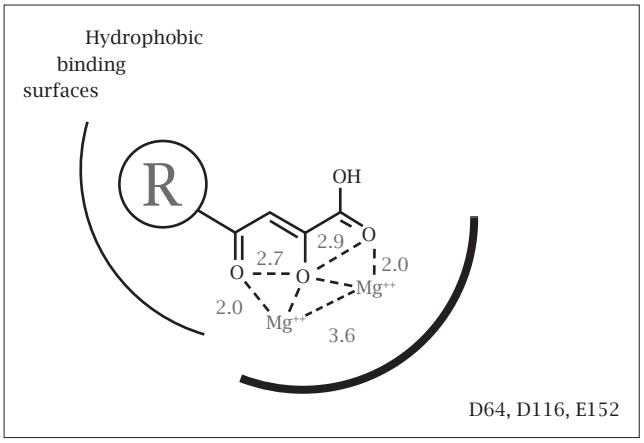
(LTR) regions at each end of the fully reverse transcribed HIV-1 DNA. In the context of this complex (termed pre-integration complex or PIC), integrase then catalyses the subsequent enzymatic reactions, *3' end processing* which removes the terminal 3' dinucleotide from each end of the viral DNA and *strand transfer* which results in the covalent linkage of the viral DNA and the host DNA.[5] All integrase inhibitors in clinical development to date specifically target the strand transfer reaction[6-8] and are thus alternately referred to as either integrase inhibitor (INIs) or more specifically integrase strand transfer inhibitors (InSTIs). In the context of the viral infection process, inhibition of integration results in an irreversible block to HIV-1 replication as the unintegrated viral DNA is subject to metabolism by a variety of cellular enzymes (Figure 1). Although most of the unintegrated viral DNA is degraded, recombination and repair processes in the cell can also generate 1 and 2 LTR circular DNA byproducts. These circles were first described with integration defective HIV-1 viruses,[9,10] but are now a hallmark of the effect of integrase inhibitors both *in vitro* and *in vivo*.[11,12]

The biochemistry of integrase inhibitors

I*ntegrase strand transfer inhibitors* or InSTIs have minimal effects on either the assembly of the integrase DNA complex or the 3' end processing reaction. Their selective effect on the strand transfer reaction is a direct result of a now well-defined mechanism of action in which the inhibitor: 1) binds only to the specific complex between integrase and the viral DNA and not to integrase in the absence of the DNA, and 2) interacts with the two essential magnesium metal ion cofactors in the integrase active site post assembly.[13] All InSTIs therefore have two essential components to their chemical structure, a metal binding pharmacophore which sequesters the active site magnesium and a hydrophobic group which interacts with the viral DNA as well as the enzyme in the complex. (See model in Figure 2) The metal binding portion of these compounds is absolutely essential for inhibition while the hydrophobic component of the chemical structure is largely responsible for enhancing the overall affinity and specificity of the inhibitor in the integrase DNA complex.[14] The recent co-crystallization of the Foamy virus integrase DNA complex or intasome with both RAL and EVG[15] corroborates many of the original biochemical observations that led to this model and provides a structural basis for understanding the breadth of antiviral activity that has been observed for InSTIs across all HIV-1 subtypes as well as other retroviruses including HIV-2 and XMRV.[16-22] In the co-crystal structure the general architecture and amino acids within the active site of the Foamy virus intasome are highly conserved among retroviral integrases, as are the immediate surrounding interactions with InSTIs.

Figure 2: Model of InSTI interactions in the active site of integrase (based on Grobler, Stillmock *et al.*[14]).



The striking conservation of the active site interactions in the intasome would be consistent with the observation that mutations which engender resistance to InSTIs do not appear to be present as polymorphisms in the HIV-1 *quasispecies* at baseline in integrase inhibitor *naïve* patients.[23-25] Baseline resistance to RAL has not been detected in several studies including an analysis of both B and non-B subtypes. Primary InSTI mutations have also not been detected in InSTI-*naïve* individuals, regardless of exposure to other antiretroviral agents or duration of HIV-1 disease. However, naturally occurring InSTI polymorphisms are present for integrase as for all HIV-1 proteins, particularly in patients infected with non-B viruses.[19] The clinical consequence of integrase polymorphisms on InSTI response and resistance remains to be determined but limited data to date has suggested no difference in overall clinical response to RAL among B-and non-B HIV-1 infections.[18] Studies that have evaluated *in vitro* the susceptibility to RAL and EVG using large panels of clinical isolates with multiple HIV-1 subtypes have also shown that fold changes in the IC^{50} to these inhibitors are below the biological threshold.[26] In addition, HIV-1 group O and HIV-2 viruses, which show significant heterogeneity in the integrase gene compared to group M viruses display similar susceptibility to RAL.[24]

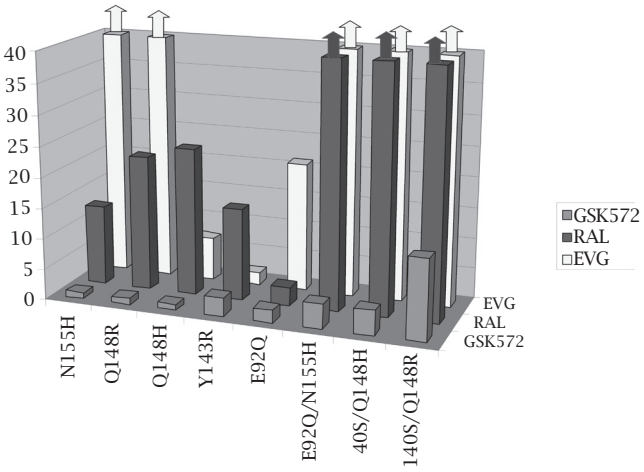
The common mechanism of action and conserved binding mode for InSTIs also has important implications for understanding cross resistance to the class. In principle, resistance mutations can either affect interactions between the metal binding pharmacophore and magnesium at the integrase active site and/or directly affect interactions between the pendant groups in the inhibitor and the enzyme and viral DNA.[8] Mutations which engender resistance to InSTIs almost always map within the integrase active site near the amino acid residues that coordinate the essential magnesium cofactors[27,15] rather than by affecting interactions with the enzyme viral DNA. Given the critical nature of the metal cofactors to integrase function, these mutations have a deleterious effect on enzymatic activity and viral replication. However, these mutations also engender significant cross resistance and although different InSTIs have distinct resistance mutations there is significant overlap in resistance to many of these inhibitors.[28,29]

InSTI resistance *in vitro* and *in vivo*

The development of resistance to structurally diverse InSTIs has now been documented both *in vitro* and *in vivo*.[11, 28, 30-37] *In vitro*, the selection of resistance has been shown to require multiple passages of HIV-1 in cell culture, likely as a result of both the sequential accumulation of mutations as well as the reduced fitness of these mutants.[30,31] While diverse InSTIs can select different mutations, nearly all of the amino acid residues associated with InSTI resistance localize within the integrase active site proximal to the amino acid residues involved in coordinating the metal cofactors consistent with a common mechanism of a metal sequestration.[8] Importantly, resistance to InSTIs does not affect susceptibility to other antiretroviral agents, including PIs, NNRTIs, RTIs and the various classes of entry inhibitors.

The selection of resistance to early, prototypic integrase inhibitors and clinical development candidates has identified a variety of genetic pathways which are defined by a single characteristic or signature resistance mutation.[38,39] In clinical trials three primary mutational routes conferring high-level resistance to RAL have been observed: 1) N155H in combination with L74M, E92Q or G163R, 2) Q148H/R/K with E138K or G140S/A and 3) Y143R/C mutation plus other mutations.[40] For RAL, these general patterns have been confirmed in a variety of cohort studies.[41,42] For EVG, patients experiencing treatment failure also selected viruses with mutations at E92Q, E138K, Q148H/R/K, and N155H as well as other mutations.[32,33] *In vitro,* other InSTIs have been shown to select different mutations including S153Y.[27] While the overall magnitude of the effect of any specific individual mutation can vary for different InSTIs there is considerable overlap between the resistance profiles among InSTIs including all three of the current clinical candidates (Figure 3).

Figure 3: Resistance and cross resistance among InSTIs (RAL, EVG and GS5172). The effect of RAL resistance associated mutations (RAMS) as site directed mutations on antiviral activity in vitro.

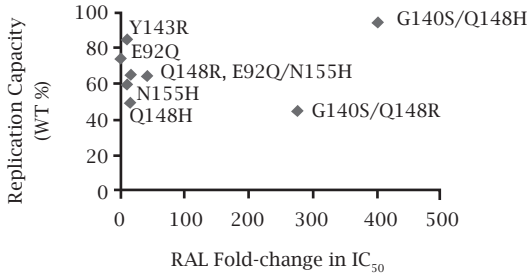


For RAL, the N155H mutation confers ~10-fold loss of sensitivity, while the Q148H, Q148K, and Q148R mutations confer ~20 to 40-fold resistance. Among other single amino acid changes tested, only Y143R conferred > 10-fold resistance to RAL. All other substitutions (E92Q, T97A, V151I, G140A, G140S) have significantly smaller effects (< 4-fold). Because of the frequency with which they have been observed both in clinical trials and in cohort studies and the observation that each of these mutations individually confers a > 10-fold loss of RAL susceptibility, mutations at Y143, Q148, and N155 are considered "primary mutations;" whereas other mutations which confer limited change in susceptibility to RAL and are observed almost exclusively in the context of these "primary" substitutions are considered to be "secondary" mutations. It should be noted however, that there may be additional context effects which contribute to resistance and the magnitude of the effect of both single and multiple InSTI mutations can vary in clinical isolates.

The evolution of secondary mutations in the context of a primary mutation has been shown to increase the level of overall resistance to RAL both in the context of site directed mutants as well as in clinical isolates[40] (Figure 4). The addition of L74M, E92Q, T97A, Y143H, E92Q+T97A, V151I, or G163R to N155H increases the fold-change IC50 to RAL from approximately 10-fold (N155H alone) to as much as more than 100-fold (range: 20 to > 100-fold). The addition of a G140 mutation to Q148R or H augments resistance in a surprisingly specific manner, G140S/Q148R and G140S/Q148H exhibit ~405-fold and ~521-fold resistance, respectively.

Given the highly conserved nature of the amino acid residues associated with primary resistance to RAL, it is not surprising that viruses with these mutations exhibit reduced replication capacity (40 to 60% of wild-type) (Figure 4). However, in contrast to the observation that secondary mutations generally augment the level of resistance to RAL when combined with primary mutations, the effect of secondary mutations on the replication capacity viruses of viruses with primary InSTI mutations can vary considerably. Although in most cases, secondary mutations have either no effect or further reduce replication capacity, in the most striking case the addition of the G140S secondary mutation to Q148H compensates for the replication capacity defects of Q148H (Figure 4). The latter likely accounts

Figure 4: Effects of resistance mutations on RAL activity and viral replication capacity as measured in a single cycle HIV-1 infectivity assay.



for the observation that both in clinical studies and in surveillance studies, G140S/Q148H is the most frequent combination observed in patients failing RAL with bona fide resistance.

In patients with virologic failure and RAL resistance, viruses with Q148H mutations are fitter than those with N155H mutations.[43] Longitudinal genotyping has shown that when RAL resistance evolves with the Q148H mutation, the integrase genotype is stable over time even when patients are maintained on RAL. In contrast, N155H viruses are frequently replaced by viruses with Q148H mutations likely present in the *quasispecies* early on during failure. The N155H and Q148H mutations have been shown to occur on separate viruses in these patients. The ultimate emergence of a dominant Q148H population from such mixtures demonstrates that in the presence of RAL, Q148H variants have a competitive advantage relative to N155H mutants due to the significant difference in replication capacity between these the two pathways.

Though replication capacity may play a role in pathway selection the acquisition of InSTI mutations in RAL failures is mainly driven by selection for higher levels of resistance. After virologic failure, the overall level of resistance to RAL tends to increase with time.[41] In many patients, the number of integrase resistance mutations increases with time correlating with higher-level resistance. In other patients, switching from the N155H pathway (generally lower-level resistance) to the Q148/K/R variants associated with higher-level resistance was observed. The replication capacity of viruses with Q148R or K plus secondary mutation(s) is similar to that of viruses with N155H plus secondary mutation(s), therefore switching of the population in these patients is best explained by selection pressure requiring the higher level of resistance.

Clinical observations: virologic failure and resistance

Several recent studies have shown that a substantial proportion of virological failures in patients on RAL therapy occur in the absence of InSTI mutations.[41,42,44] Even among a cohort of highly treatment-experienced subjects failing RAL containing regimen, failure with wild-type integrase was relatively common (particularly during early failure); however, InSTI resistance may emerge in subjects who remain on RAL in the context of virologic failure. Several reports have described evolution of resistance mutations under continued RAL pressure in the absence of complete viral suppression.[39,41] In some early treatment failures, virus populations containing N155H alone were observed to switch to virus populations with Q148R or H. Additional studies have also documented the dynamics of RAL resistance and the evolution of N155N/H to Q148H or N155H to Y143R in subjects with incomplete viral suppression.[45] The observation of InSTI resistance evolution during virologic failure may suggest that remaining on a RAL regimen could mitigate future therapeutic options within the InSTI class. However, some studies have suggested RAL may have persistent immunologic and virologic benefit even after the development of resistance.[41] Given the different overall impact on viral replication capacity and distinct evolutionary trajectory for each resistance pathway, whether to continue RAL in patients who have limited options for complete viral suppression remains a challenging question that may depend as much on the specific InSTI resistance pathway as on the patient's specific clinical situation. It is interesting to note that in the Coronet study where data were collected from multiple centers throughout Europe, of the three major pathways of RAL resistance, N155H and Y143R/C were observed in both B and non-B subtypes, while Q148H/R/K was less common in non-B subtypes. Further studies are needed to determine how the development of RAL resistance through different resistance pathways may be influenced by genetic context and whether these differences will impact the overall ability to recycle current integrase inhibitors or influence response to next generation agents in the class.

SUMMARY

The first integrase inhibitor, RAL, was introduced in 1997 and is now approved for both treatment experienced and treatment *naïve* patients. A limited number of additional integrase inhibitors is now advancing in clinical development, and a wide variety of chemically diverse InSTIs has been disclosed in the recent patent and scientific literature. These inhibitors exhibit a common mechanism which involves binding to the active site magnesium in the integrase DNA complex. Owing to the common manner in which InSTIs engage the magnesium within the integrase active site, overlapping resistance is observed with many compounds in this class and there is substantial cross-resistance among the first generation agents RAL and EVG *in vitro* and *in vivo*. In HIV-1 infected patients resistance to RAL can evolve through multiple independent genetic pathways characterized by signature mutations at one of three active site residues (S143, N155 and Q148) and the stepwise accumulation of secondary mutations which lead to high level resistance. Although RAL and InSTIs in general are active across diverse HIV-1 subtypes[46] and a broad range of retroviruses including HIV-2 and XMRV, additional work is needed to understand the development of resistance in diverse HIV-1 genetic backgrounds and context effects which may influence the evolution of InSTI resistance. Understanding the underlying mechanisms of resistance and the recent co-crystallization of RAL and EVG with the Foamy virus intasome will be useful for future drug development efforts aimed at identifying novel InSTIs that can address the emergence of resistance to first generation InSTIs in HIV-1 patients.

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