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# HIV Type 1 Integrase Inhibitors: From Basic Research to Clinical Implications

*Oyebisi Jegede[1], John Babu[2], Roberto Di Santo[3], Damian J. McColl[4], Jan Weber[5] and Miguel E. Quiñones-Mateu[5]*

*[1]Department of Cellular and Molecular Biology, Kent State University, Kent, Ohio, USA; [2]Panvirex LLC, Cleveland, Ohio, USA; [3]Pasteur Institute, Cenci Bolognetti Foundation, Department of Chemistry and Technology of Drug Pharmacology, Sapienza University of Rome, Rome, Italy; [4]Gilead Sciences, Foster City, California, USA; [5]Diagnostic HYBRIDS, Inc., Cleveland, Ohio, USA*

## Abstract

***Similar to other retroviruses, productive infection with HIV-1 requires three key steps in the viral replication: (i) reverse transcription of viral genomic RNA into viral cDNA by the viral reverse transcriptase; (ii) integration of viral cDNA into host cell genome using the viral integrase; and (iii) cleavage of newly synthesized viral polypeptide by the viral protease into individual viral proteins during new virion assembly. Following their discovery, all three viral enzymes were considered as targets for antiretroviral drugs. However, while multiple reverse transcriptase and protease inhibitors have been used for more than 12 years to treat HIV-infected individuals, only recently has the viral integrase enzyme emerged as an alternative, clinically validated target to block HIV-1 replication. Here we review the biology of HIV-1 integration, the mechanisms of action and development of resistance to integrase inhibitors, and the latest data on the most recent clinical trials involving this promising, novel class of antiretroviral drugs.*** **(AIDS Rev. 2008;10:172-89)**

*Corresponding author: Miguel E. Quiñones-Mateu, quinones-mateu@dhiusa.com*

## Key words

***HIV. Integrase inhibitors. Drug resistance. Replicative fitness.***

## Novel targets for antiretroviral therapy

HIV/AIDS has evolved into an ongoing global pandemic with significant socio-economic impact. Currently, 23 antiretroviral drugs are approved for the treatment of HIV-1 infection, the majority of which target two essential viral enzymes: reverse transcriptase and protease. Reverse transcriptase inhibitors (RTI) can be subdivided into two classes, based their distinct mechanisms of action, and include nucleoside/nucleotide reverse transcriptase inhibitors (NRTI) and nonnucleoside reverse transcriptase inhibitors (NNRTI). Several RTI have been combined into fixed-dose combination tablets, which contain either two or more NRTI, or two NRTI and the NNRTI efavirenz. The protease inhibitors (PI) comprise the third class of approved antiretroviral drugs, all of which inhibit the essential proteolytic processing of viral proteins. Other classes of drugs act extracellularly to prevent the entry of the virus into the host cell (fusion or entry inhibitors). Enfuvirtide (T-20, Fuzeon™, Roche Laboratories Inc. and Trimeris, Inc. USA), a fusion inhibitor, was introduced in 2003, and acts to mimic the viral gp41 polypeptide, thereby blocking the fusion of the viral and cellular membranes. Maraviroc (UK-427857, Selzentry™, Pfizer Inc. USA) a newly approved member of the entry inhibitors class, targets coreceptor binding by HIV-1 (CCR5 antagonist).

**Correspondence to:**
Miguel E. Quiñones-Mateu
Diagnostic HYBRIDS, Inc.
7100 Euclid Avenue, Suite 190
Cleveland, Ohio 44106, USA
E-mail: quinones-mateu@dhiusa.com

For the last decade, combination therapy, known as highly active antiretroviral therapy (HAART), has been the gold standard of care for HIV-1 infected individuals in most developed countries. HAART has been credited with a highly significant reduction in HIV/AIDS mortality by reducing plasma viremia, increasing $CD4^+$ lymphocytes count, reducing immune activation, and restoring lymph node architecture[1-7]. On the other hand, many currently available antiretroviral drugs have also been associated with long-term side effects[8], inability to eradicate latent reservoirs of HIV-1[9-11], development of drug resistance, and eventual failure of therapy[12,13]. Moreover, new infections with HIV-1 strains exhibiting multiclass drug resistance, together with the continual evolution of drug-resistant virus strains[9,14,15], highlights the urgent need to develop novel antiretroviral drugs, preferably against new HIV-1 targets.

Several potential targets for the development of new antiretroviral drugs have been identified due to the substantial increase in the knowledge of the structural biology of HIV-1 and its interaction with host cells[16]. Among the viral targets being evaluated for new drug development, the most exciting opportunities currently under consideration include (i) viral entry, with a major focus on blocking the interaction of the virus with its two major coreceptors, CCR5 and CXCR4, (ii) integration of viral DNA into the human genome, and (iii) maturation of the viral particle. The use of different compounds to block the interaction of the viral envelope with its major receptor (the CD4 protein) or coreceptors (CCR5 or CXCR4) is relatively advanced and, as a consequence, has been extensively reviewed[17,18]. Inhibition of virion maturation, that is, the blockage of the cleavage of Gag (p55) and Gag/Pol (p160) precursor polyproteins into structural proteins and enzymes (i.e. protease, reverse transcriptase, and integrase), represents another intriguing opportunity to develop antiretroviral drugs. Although viral maturation inhibitors are less advanced in clinical development than protease, reverse transcriptase and entry inhibitors, the first member of this drug class (PA-457, bevirimat) has been shown to reduce plasma viral RNA load by > 1 $\log_{10}$-fold in phase IIa clinical trials[19].

The HIV-1 integrase inhibitors act at a point in the viral lifecycle following classical antiretroviral drugs such as NRTI, NNRTI and the most recently developed entry inhibitors, but prior to the effect of PI. Viral integration is a particularly desirable target because, like other retroviruses, HIV-1 requires integration into the host genome for its replication and propagation[20]. Typical products of the HIV-1 integration process include linear and nonintegrated DNA, which are degraded in cells within 24 hours, plus 1- and 2-LTR circles (formed by the ligation of the long terminal repeat ends of the linear HIV-1 genome)[21-23]. Most importantly, HIV-1 integrase has no known human equivalent and offers the possibility of high drug specificity with limited cellular toxicity. This review will focus on the viral integrase and the development of integrase inhibitors as new anti-HIV-1 therapeutic agents.

## Biology of HIV-1 integration

### *Structure and function of the integrase*

HIV-1 integrase is an essential viral enzyme that is required to catalyze the specific and efficient splicing of the viral DNA product of reverse transcription into the host cell genomic DNA[24-26]. The 32 KDa integrase enzyme is a 288 amino acid protein encoded by the 3′-end of the *pol* gene and approximately 50-100 copies of the integrase enzyme are packaged per virion particle[27]. Like other viral proteins, the mature functional integrase enzyme is generated by proteolysis of the precursor Gag-Pol fusion protein by the viral protease enzyme[28]. Integrase consists of three structural and functional domains (i.e. an N-terminal domain, a catalytic core domain, and a C-terminal domain) and the functional integrase enzyme is composed of integrase homodimers that are proposed to further associate with each other to form a multimer complex in solution[29,30] (Fig. 1). Crystal structures of several integrase catalytic core domains, obtained as dimers or trimers[31-34], show that it consists of five β-sheets flanked by six α-helices that are connected by flexible loops.

The N-terminal domain of the integrase (amino acids 1-50) is characterized by two pairs of highly conserved histidine and cysteine residues (histidine residues 12 and 16; cysteine residues 40 and 43) that form a “HH-CC” or zinc finger motif that involves the chelation of one zinc atom per integrase monomer[35,36]. The N-terminal domain is required for high-order multimerization that is stimulated by zinc[36]. A zinc atom is required to stabilize the folded structure of the N-terminal domain of HIV-1 integrase and is necessary for optimal enzymatic activity[35,37].

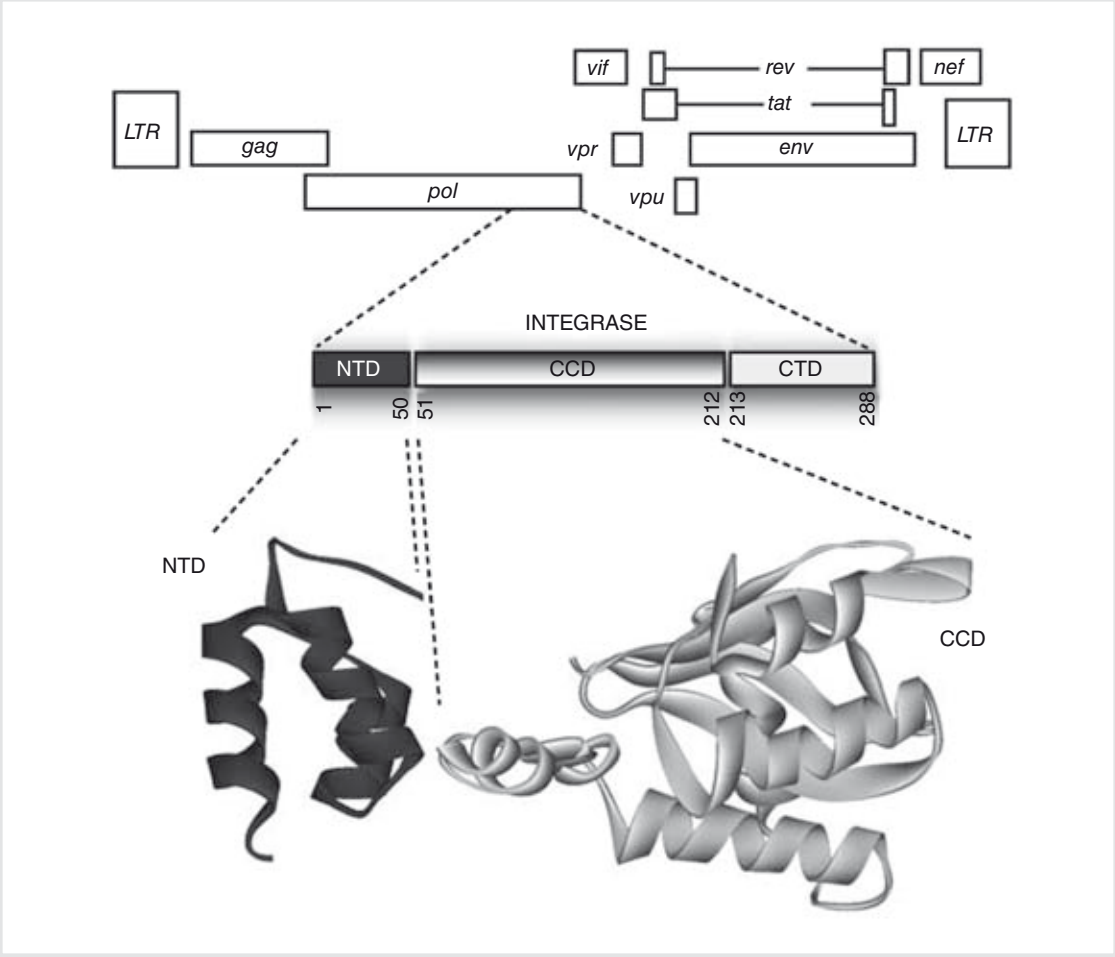



**Figure 1.** *HIV-1 integrase structure and functional domains. NTD: N-terminal HH-CC zinc finger domain (binds cellular factors); CCD: central catalytic domain (binds $Mg^{2+}$, $Mn^{2+}$); CTD: C-terminal domain (binds DNA non-specifically); LTR: long terminal repeat.*

The catalytic core domain includes amino acids 51-212 of HIV-1 integrase. The catalytic core domain forms a dimer in solution and in the functional enzyme; the monomer structure of the catalytic core is similar to the folds of RNaseH and the catalytic domains of Holliday junction resolvase RuvC and MuA transposase[38,39]. These enzymes belong to the DNA processing polynucleotide transferase superfamily that cut and join DNA by direct transesterification[40-43]. The catalytic core domain is required for the cleavage and formation of phosphodiester bonds[44]. The active site of the catalytic core domain contains three highly conserved acidic amino acids forming a catalytic triad (i.e. residues D64, D116, and E152, also known as the D, D E motif), which are absolutely essential for efficient integrase enzymatic activity[45]. Each one of these amino acids is required for the catalysis of the three steps of viral integration into host DNA; substitution of any of these residues significantly reduces the enzymatic activity of HIV-1 integrase[46-48]. Interestingly, although the catalytic core domain can also catalyze the disintegration reaction (the reverse of integration) by itself, the N-terminal and C-terminal domains of integrase are also required for the 3′ processing and strand-transfer reactions[40,45,49].

The C-terminal domain (amino acids 213-288) is the least conserved of the three domains, as shown by a relatively weak sequence homology in the C-terminal domain among retroviral integrases. The HIV-1 C-terminal domain shares some structural homology

with the SH3 class of DNA-binding domain, can bind DNA nonspecifically, and is required for both the 3′ processing and strand-transfer activities of HIV-1 integrase[30,49].

### *Process of HIV-1 integration into the host genome*

Integration of HIV-1 DNA into any point of the host chromosomal DNA is required for the persistence of HIV-1 infection through latent anatomical and cellular viral reservoirs[8,14,15]. Following reverse transcription of the viral genomic RNA, the resulting double-stranded DNA intermediate undergoes two reactions catalyzed by integrase. In the cytoplasm, the HIV-1 integrase recognizes and binds to a specific, imperfect, and inverted sequence in the long terminal repeats (LTR) of the reverse-transcribed DNA[44,50]. It then catalyzes the removal of a GT dinucleotide immediately 3' to the conserved CA dinucleotide at the 3' end of both strands of the viral cDNA (i.e. at the U3 and U5 LTR ends) by a nucleophilic attack on the phosphodiester bond between the deoxyguanosine and deoxyadenosine[51,52]. This initial step is called 3' processing and the integrase remains bound to the LTR, forming a preintegration complex (PIC). The PIC contains viral proteins (including, matrix, Vpr, p7/nucleocapsid, reverse transcriptase[53,54]) and the newly transcribed viral DNA, and also contains host proteins (including barrier to auto-integration factor, interactor 1, lens epithelium derived growth factor, heat shock protein-60, and high-mobility group protein A1[55-61]). The PIC is actively transported to the nucleus, where the second reaction called 3'-end joining or strand transfer occurs.

The strand-transfer reaction consists of a direct nucleophilic attack on the host chromosome (acceptor DNA) by the 3'-hydroxyl recessed viral DNA ends (donor DNA). Both ends of the viral DNA, which are kept in close proximity, integrate at the 5'-ends of the host chromosomal DNA with a 5-base pair stagger[44,62,63]. The two unpaired nucleotides at the 5'-ends of the viral DNA are removed, and the gaps at the integration site on both the termini are filled, probably by cellular repair enzymes, as gaps seem not to be repaired in the presence of PIC proteins *in vitro*[64]. The strand-transfer reaction completes the viral DNA integration into the host chromosome. The integrated viral DNA, now termed a provirus (proviral DNA), becomes the template for new virion synthesis, a process accomplished by host cellular machinery. A single provirus is sufficient for the production of thousands of new virions from an HIV-infected cell. Although each step in the viral lifecycle (reverse transcription, integration, and protease cleavage) are essential for viral replication, a single provirus can function as a production house for synthesis of new virions, with resulting amplification of the viral infection, and therefore makes the viral integration step a central target for antiretroviral drug development.

## Development and mechanism of HIV-1 integrase inhibitors

For many years, the consensus has been that antiretroviral drugs against HIV-1 integrase would be a valuable complement to approved RTI and PI for antiretroviral therapy. However, there were several concerns relating to the capacity for integrase inhibitors to be useful in limiting viral replication *in vivo*. For example, numerous copies of the integrase enzyme enter the cell with the infecting virus and only two integration events are required to form a provirus. Thus, it was felt that the integrase could be a difficult target to inhibit, thereby reducing the potential therapeutic value of integrase inhibitors *in vivo*. Such concerns have now been shown to be unfounded, with the relatively recent identification of a class of compounds that unambiguously inhibits HIV-1 replication by targeting the integrase enzyme[62,65,66]. These inhibitors demonstrated a much higher affinity for integrase in the presence of viral DNA as compared to the purified integrase enzyme alone and demonstrated selectivity for the strand-transfer reaction, thus attesting to the value of these inhibitors in preventing HIV-1 replication[44,62].

### *Developments in integrase inhibitors*

Promising lead compounds that would serve as a pharmacophore base for integrase inhibitor development have been discovered in recent years. As in many drug discovery programs, random chemical library screening resulted in the initial identification of integrase inhibitors. Scientists at Merck Research Laboratories paved the way for the development of this drug class following the identification, by random chemical library screening, of molecules having a

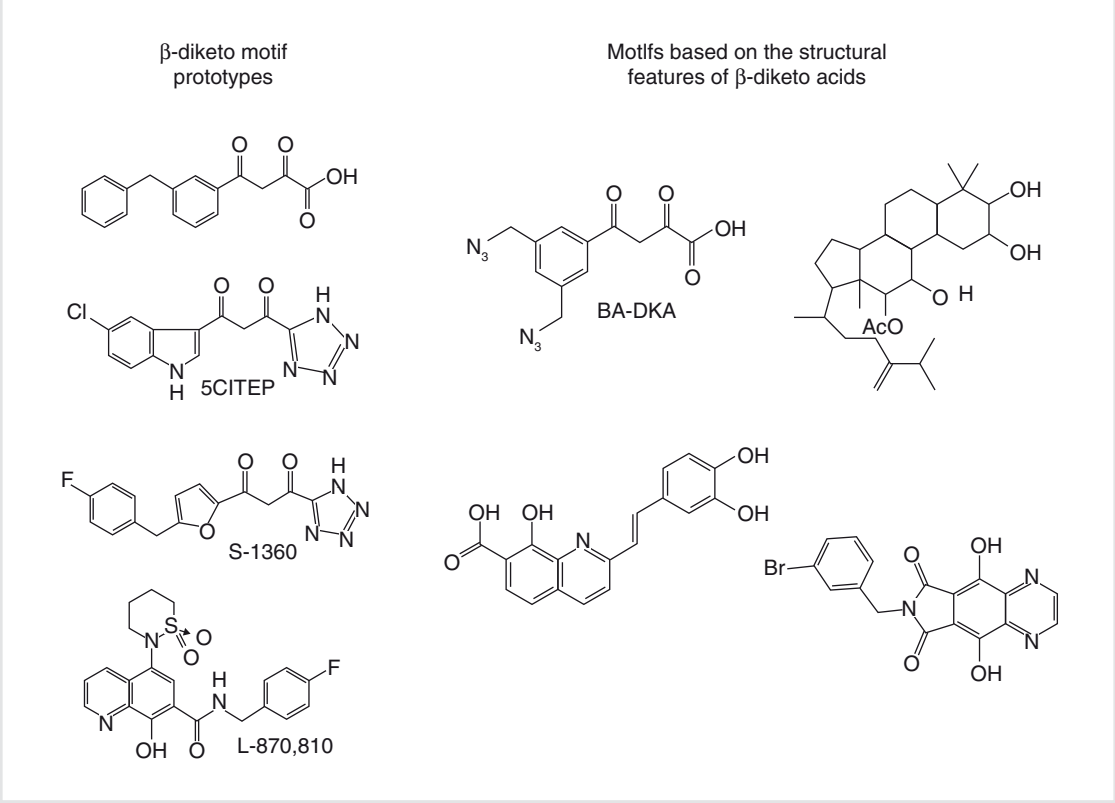



**Figure 2.** β*-diketo motifs as prototypes for novel compounds aimed to inhibit HIV-1 integration.*

β-diketo acid motif[31,66]. In fact, the β-diketo acid motif became a prototype for many of the compounds that were developed subsequently (Fig. 2). For example, the structural features of β-diketo acids as integrase inhibitors were further explored in the development of other integrase inhibitor compounds containing diazide or styrylquinoline motifs[67], other motifs such as catechol-containing catesteroid[68-70], a naphthylazo-containing compound[71,72], and other classes of compounds such as phthalimide and uracil derivatives (Fig. 2).

## *Mechanisms of integrase inhibitors*

Unlike competitive inhibitors that simply compete for substrate binding, integrase inhibitors that block the strand-transfer reaction are catalytic inhibitors. In general, integrase inhibitors can be classified as those that target (i) both the 3' processing and strand-transfer reactions (i.e. bi-functional inhibitors) and (ii) compounds that inhibit the strand-transfer reaction alone (i.e. strand-transfer-specific inhibitors). Based on several structural activity relationship studies, it was established that integrase inhibitors bind to distinct regions of the integrase enzyme following a conformational change induced upon donor DNA binding, and then impair integrase enzyme function by interaction with the catalytic triad. For example, styrlquinoline derivatives inhibit only the 3'-processing reaction but not the strand-transfer reaction[73]. Other β-diketo motif-containing compounds inhibit both reactions: the prototypic diketo acid integrase inhibitors L-708, 906 and L-731,988 inhibited the strand-transfer reaction but did not inhibit 3' processing; on the other hand, another diketo acid-containing compound, 5CITEP, inhibited both reactions. Bifunctional inhibitors may contact both the donor and target DNA binding sites, whereas the strand-transfer inhibitors may bind selectively to

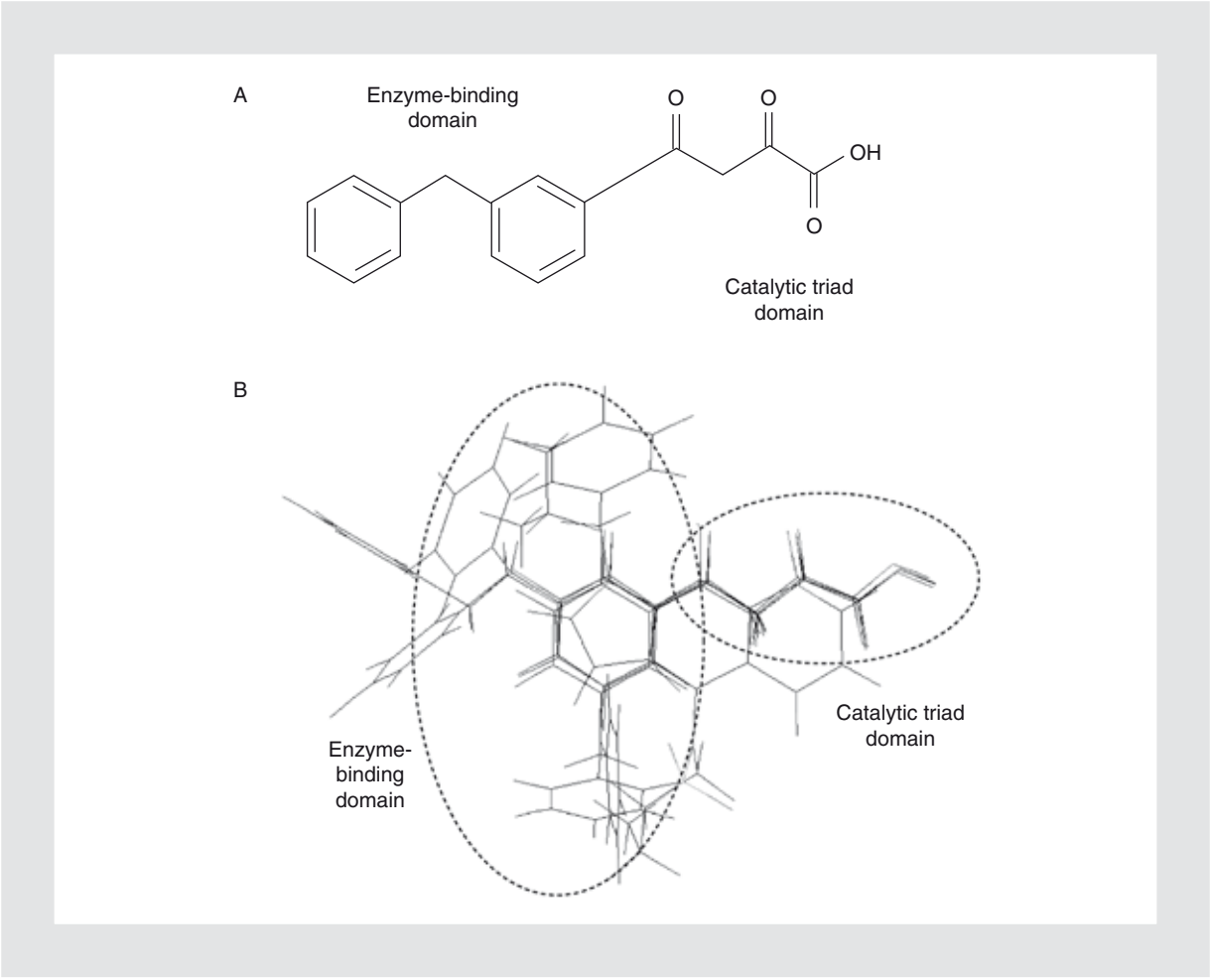



**Figure 3.** *Pharmacophore analysis of HIV-1 integrase inhibitors. (***A***) Common structural features of integrase inhibitors. (***B***) Molecular topology of integrase inhibitors.*

the target DNA binding site[74]. More important, strand-transfer inhibitors recognize a conformation of the integrase active site that is defined only after assembly on a specific viral DNA end, a mechanism akin to allosteric inhibition. Although the potential advantages of these different integrase inhibitor classes are not yet fully understood, it is anticipated that strand-transfer inhibitors will demonstrate greater selectivity and specificity for the viral integrase than 3'-processing inhibitors.

## *Pharmacophore analysis of integrase inhibitors*

As described above, integrase inhibitors include compounds that exhibit a broad structural diversity. However, the different compounds seem to follow a common pattern: almost all the compounds identified contain metal-chelating motifs. Based on pharmacophore analysis of several integrase inhibitors, their structural features can be divided into two domains: (i) the enzyme-binding domain and (ii) the catalytic triad domain, as shown in figure 3A. The enzyme-binding domain facilitates and potentially stabilizes inhibitor binding to the integrase enzyme, thereby allowing the catalytic triad domain to be presented to the catalytic triad.

The enzyme-binding domain is highly flexible, accommodates aromatic hydrophobic group(s), and can tolerate aromatic moieties of various sizes[75]. The enzyme-binding domain of the inhibitors can be viewed as "anchoring residues" that binds to the complementary region in the integrase enzyme (corresponding to

the "inhibitor binding" region). In addition to the anchoring role, the hydrophobic residue of the enzyme-binding domain of the integrase inhibitor probably also facilitates the transport of the integrase inhibitor across the cell membrane. The structural flexibility of the enzyme-binding domain allows the attachment of additional hydrophobic residues that further facilitate cell membrane permeability as well as enhance binding of the molecule to the hydrophobic regions of integrase.

In contrast to the highly flexible structural features of the enzyme-binding domain, the catalytic triad domain of the integrase inhibitor is highly conserved and shares a common motif. A general feature of this domain is that they all contain divalent metal ion chelating motifs such as catechol, 1,2-diols, β-dicarbonyls, α-hydroxy acids, or quinolinols. Another common feature of most of the integrase inhibitors identified to date is that the catalytic triad domain is in a planar configuration with the enzyme-binding domain. The molecular topology of selected integrase inhibitors that differ in their enzyme-binding domain but are overlaid on their catalytic triad domain is shown in figure 3B to illustrate the plasticity of integrase inhibitors in binding to the integrase catalytic site.

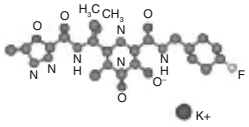
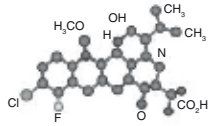
All available pharmacologic and biological data obtained with different integrase inhibitors indicate a common mechanism of action. Accumulating evidence suggests that integrase inhibitors sequester the divalent metal ions from the active site[67-70,73,74] and inhibit enzymatic catalysis. Since divalent metal ions are crucial for integrase catalysis, and mutation of the catalytic triad residues significantly impairs integrase catalysis, the metal-chelating mode of action is consistent with the notion that all integrase inhibitors act as catalytic inhibitors. The present notion, based on several structural activity relationship studies[66-70,74,76,77], is that integrase inhibitors exert their antiviral property by chelating $Mg^{2+}$ metal ions at the active site. The metal-chelating mechanism of integrase inhibitors can be further understood from the characterization of integrase inhibitor-resistant HIV-1 mutants.

## Clinical trials of HIV-1 integrase inhibitors

Preclinical studies and clinical trials on integrase inhibitors started in the mid 1990s following the discovery of the first representative inhibitors. Aronex Pharmaceuticals developed AR-177 as a promising integrase inhibitor; however, further studies showed that this drug did not actually interfere with DNA integration and the related clinical trials were discontinued due to unsatisfactory results[78-81]. Studies with the integrase inhibitor S-1360[82], developed by Shionogi/GlaxoSmith-Kline, were discontinued due to poor bioavailability. Merck's naphthyridine derivative L-870,810 was the first integrase inhibitor proven to be effective as an antiretroviral agent in HIV-1-infected individuals. In a phase I proof-of-concept study, two different doses (200 and 400 mg) of L-870,810 given as a 10-day monotherapy in untreated HIV-infected subjects resulted in a > 1.5 $\log_{10}$ decrease in plasma HIV-1 RNA levels[83]. Unfortunately, the development of this compound was discontinued due to hepatotoxicity associated with long-term dosing in animal studies.

These initial studies led to the subsequent development of two integrase inhibitors, which showed great promise as the leaders of this new class of antiretroviral drugs. The first one is L-900,612 (MK-0518, raltegravir, Isentress®, Merck & Co., Inc. USA) a potent pyrimidine derivative ($IC_{95}$ = 33 nM) related to the original L-870,810 compound developed by Merck. Among other relevant properties, this derivative has good bioavailability in uninfected subjects (it is metabolized primarily via glucuronidation, UGT1A1) and shows weak inhibition or induction of CYP3A4 (Tabla 1). A multicenter, double-blind, randomized, placebo-controlled two-part phase II study, with patients randomized 1:1:1:1 to received either one of four doses of raltegravir (100, 200, 400, and 600 mg twice daily) vs. placebo for 10 days of monotherapy, showed promising results[84]. Thirty-five treatment-naive patients with plasma HIV-1 RNA levels ≥ 5,000 copies/ml and $CD4^+$ T-cell counts ≥ 100 cells/$mm^3$ were treated with raltegravir or placebo. Following 10 days of treatment, the mean HIV-1 RNA decrease was 1.7 to 2.2 $\log_{10}$ across different arms, and the proportion of subjects with < 400 copies/ml or < 50 copies/ml was 50-70 or 13-29%, respectively. Raltegravir was well tolerated with no dose effect observed even at the maximum dose of 600 mg twice daily. The most common side effects were headache, fatigue, and dizziness, and these were comparable in frequency and severity to those observed in the placebo control group. There were no serious adverse events, and no participants discontinued the study because of side effects[84]. Moreover, pharmacokinetic parameters at day 10 showed that plasma concentrations greater than $IC_{95}$ values were attained in the majority of patients.

**Tabla 1. A comparison of the two lead integrase inhibitors: raltegravir (MK-0518, Isentress™) and elvitegravir (GS-9137)**

| | Raltegravir (MK-0518, Isentress ) | Elvitegravir (GS-9137) |
|---|---|---|
| **Chemistry** | | |
| Structural formula |  |  |
| Molecular formula | C20H20FKN6O5 | C23H23ClFNO5 |
| Molecular weight | 482.51 | 447.9 |
| **Clinical trials** | | |
| Clinical development | Approved by the FDA (U.S.) | Phase III |
| Metabolism | Glucuronidation | CYP3A4 ( primarily) & gluc uronidation |
| Interaction with other antiretroviral drugs | Unlikely | None, except with ATV/r or LPV/r |
| Ritonavir boosting required | No | Yes |
| Dosage form/administratio | Orally/with or without food (in study) | Orally/with food |
| Recommended daily dose | Twice daily. Dose of 400 mg is approved by the FDA (U.S.) | Once daily. Dose of 150 mg to be used in phase III studies |
| Virologic response | - 2 log10 plasma HIV RNA at week 16 - 2 log10 plasma HIV RNA at week 16 | |
| Resistance (mutations) | Q148R/H/K, N155H, Y143C/R E92Q, Q148R/H/K, N155H | |
| Side effect | Mild: upset stomach, headache, tiredness, itching, diarrhea, constipation, flatulence, and sweating | Mild: loss of appetite |

Based on these promising results, the second part of the phase II study, a dose-ranging 48-week clinical trial of raltegravir (100, 200, 400, or 600 mg twice daily) vs. efavirenz (600 mg one daily) in a combination regimen with tenofovir/lamivudine, was initiated in treatment-naive HIV-1-infected individuals. At 24 weeks, all groups showed > 2.2 $\log_{10}$ decline in HIV RNA and similar increases in $CD4^+$ T-cells (75 to 135 cells/$mm^3$), with most of the reduction in plasma viral load taking place in the first 4-8 weeks of treatment. Interestingly, the raltegravir combination (at all doses) showed a more rapid initial reduction in plasma viral load when compared with the control group treated with efavirenz, which often is used as a first-line treatment in newly diagnosed HIV-infected individuals. As with the first part of the phase II study, drug-related clinical adverse experiences were generally mild and similar in all groups. Only one subject in the raltegravir 600 mg group discontinued due to an increased aspartate aminotransferase/alanine aminotransferase ratio (AST/ALT). Thus, this preliminary analysis showed that the combination of raltegravir with tenofovir and lamivudine has potent antiretroviral activity and was generally well tolerated in antiretroviral-naive patients[85].

A similar multicenter, double-blind, phase II study comparing raltegravir (200, 400, or 600 mg orally dosed twice daily) vs. placebo, both in combination with optimized background therapy (OBT), was conducted in antiretroviral treatment experienced patients[86]. Inclusion criteria included plasma viral load > 5,000 copies/ml and $CD4^+$ T-cells > 50 cell/$mm^3$, with documented drug resistance to at least one drug in each of the NRTI, NNRTI, and PI classes. Approximately, (i) one-third of the 167 participants had inclusion of enfuvirtide in their OBT; (ii) 50% had no active drugs in their OBT, as assessed by a phenotypic sensitivity score; and (iii) more than 85% had no active PI in their OBT. Moreover, since atazanavir (a PI that inhibits the UGT1A1 enzyme) has been shown to

elevate levels of raltegravir in the blood, the study design included two sub-studies where participants either received or did not receive atazanavir. Preliminary efficacy results at week 16 highlighted the potency of raltegravir, e.g. close to 80% of the raltegravir-treated individuals, who otherwise had very limited therapeutic options in other antiretroviral classes, achieved HIV-1 RNA levels < 400 copies/ml, compared to only 20% in the placebo arm (receiving OBT only). Adverse events were similar to those observed in previous clinical trials. In all, these results showed that raltegravir was well tolerated and significantly suppressed viral replication in treatment-experienced HIV-1-infected individuals[86].

Two identical multicenter, double-blind, randomized (including blinding of the sponsor), phase III clinical trials (BENCHMRK-I and -II studies, Protocols 018 and 019, respectively) were designed to conduct two identical studies enrolling approximately 700 antiretroviral therapy experienced HIV-1-infected subjects in Europe, the Asia/Pacific region, and Peru (first group), and North, Central, and South America (second group). In each study, raltegravir was administered 400 mg twice daily vs. placebo (2:1 randomization), each in combination with an OBT. The subjects enrolled had HIV-1 RNA > 1,000 copies/ml with documented genotypic/phenotypic resistance to at least one drug in each of NRTI, NNRTI, and PI classes. Oral raltegravir 400 mg twice daily plus OBT was generally well tolerated and demonstrated potent and superior antiretroviral effect compared to placebo plus OBT, with comparable efficacy results in both studies through week 16[87,88]. In both studies, 61.8% of patients treated with raltegravir achieved HIV-1 RNA < 50 copies/ml, while 77.5% achieved < 400 copies/ml compared to 34.7 and 41.9% of patients, respectively, treated with placebo plus OBT. Interestingly, when raltegravir was combined with first use of enfuvirtide and/or darunavir, over 90% of the patients achieved plasma HIV-1 RNA levels < 400 copies/ml. Through week 48, combined analyses of both BENCHMRK studies showed that 73 and 62% of patients treated with raltegravir plus OBT achieved < 400 copies/ml and < 50 copies/ml of HIV-1 RNA, respectively, compared with 37 and 33% of patients treated with placebo plus OBT, respectively. Statistically significant differences in CD4 cell increases in favor of the raltegravir-treated patients were observed in both studies at weeks 16 and 48. Through week 48 in both BENCHMRK I and II studies, 400 mg of raltegravir dosed twice daily continued to be well tolerated in highly ART-experienced patients[87-92].

Another integrase inhibitor, a quinolinone derivative (elvitegravir, JTK-303, GS-9137), discovered by Japan Tobbaco, Inc.[93], is currently being developed by Gilead Sciences. Clinical trials of elvitegravir began in 2005 and results from phase I/II studies have been reported. Elvitegravir was demonstrated to be orally bioavailable, safe and well tolerated at once-daily doses of 100, 200, 400, or 800 mg in HIV-1 seronegative individuals, and had a good pharmacokinetic profile[94]. Kearney, et al.[95] reported more extensive pharmacokinetic and pharmacodynamic data on elvitegravir. They demonstrated that elvitegravir is metabolized by CYP3A4 and that the plasma concentration of elvitegravir can be boosted by the addition of ritonavir (Tabla 1). Elvitegravir pharmacokinetics was significantly improved when the drug was boosted with ritonavir; elvitegravir plasma half-time was increased from three to nine hours in the presence of ritonavir, thereby permitting once-daily dosing of this new integrase inhibitor[96].

A randomized, double-blind, placebo-controlled, 10-day monotherapy phase IIa study was designed to evaluate the antiviral activity, tolerability, pharmacokinetics, and pharmacodynamics of elvitegravir[97]. The study involved 40 HIV-infected treatment-naive or treatment-experienced (but currently off-treatment) patients, with plasma HIV-1 RNA levels at screening between 10,000 and 300,000 copies/ml and $CD4^+$ T-cell counts of ≥ 200 cells/$mm^3$. Elvitegravir was administered with food for 10 days at (i) 200, 400, or 800 mg twice-daily doses, (ii) 800 mg once daily, or (iii) 50 mg boosted with 100 mg of ritonavir once daily, vs. placebo. Each regimen exhibited significant, exposure-dependent (elvitegravir trough concentration) antiviral activity compared to placebo. The highest reductions in plasma viral load (approximately 2 $\log_{10}$ copies/ml) was observed with twice-daily administrations of elvitegravir at doses of 400 or 800 mg or the group dosed once-daily with 50 mg elvitegravir boosted with 100 mg ritonavir. All elvitegravir dosage regimens were well tolerated, with no serious adverse events or study drug discontinuations[97]. Pharmacokinetic and pharmacodynamic data of elvitegravir in treatment-naive and treatment-experienced patients showed that regimens of 20, 50, and 125 mg given with 100 mg of ritonavir once daily could be selected for following clinical studies[95,98].

A randomized, partially blinded, active-controlled, dose-ranging, 48-week phase II study (Study GS-183-0105) was designed to assess the non-inferiority of once-daily elvitegravir versus boosted comparator protease inhibitors (CPI/r) in HIV-infected treatment-experienced subjects. Eligible patients (n = 278) had plasma

viral load ≥ 1,000 copies/ml, one or more mutations associated with resistance to PI, and no restrictions on $CD4^+$ T-cell count. Patients enrolling in the study were highly treatment-experienced with one or more PI resistance mutations in the three elvitegravir/r and CPI/r groups. Patients were initially treated with an OBT composed of NRTI ± enfuvirtide and were randomized 1:1:1:1 (stratified by enfuvirtide use) to receive either CPI/r, or once-daily elvitegravir 20, 50, or 125 mg, all boosted with 100 mg ritonavir (elvitegravir/r). After a protocol change at week 8, patients in the elvitegravir arms were allowed to add darunavir or tipranavir when new data demonstrated a lack of drug-drug interaction with elvitegravir. The primary endpoint was the time-weighted average change from baseline in $\log_{10}$ HIV-1 RNA through week 24 ($DAVG_{24}$); however, analyses were also performed on week 16 ($DAVG_{16}$) data since only four patients receiving elvitegravir added a PI/r prior week 16. The elvitegravir/r 125 mg arm was statistically superior to CPI/r for both $DAVG_{16}$ and $DAVG_{24}$ and elvitegravir was well-tolerated[99]. Once-daily elvitegravir/r 125 mg demonstrated significant durable viral suppression in subjects with one or more active drug in OBT without boosted PI from the outset[100].

In summary, raltegravir and elvitegravir appear to be extremely potent in the clinical setting. Further studies will be needed to evaluate the long-term tolerability, safety, and the emergence of resistance *in vivo*. Moreover, the administration of these integrase inhibitors as part of first-line regimens, including drugs active against different viral targets (i.e. entry, reverse transcriptase, and protease), could limit the selection of multidrug-resistant viruses. Larger clinical trials comparing integrase inhibitor-based regimens with standard treatment will be required to validate this hypothesis. Finally, encouraging results with raltegravir and elvitegravir have led other groups to pursue the development of new integrase inhibitors. For example, GlaxoSmithKline's GSK-364735 initiated phase II studies in October 2006[101], while Bristol-Myers Squibb's compound BMS-707035 was scheduled to begin phase II clinical trials in January 2008. As was experienced with other classes of antiretroviral drugs (i.e. RTI and PI) raltegravir and elvitegravir may be only the beginning of a novel and promising series of antiretroviral integrase inhibitors.

## Resistance to HIV-1 integrase inhibitors

Preclinical development of novel integrase inhibitors, including raltegravir and elvitegravir, has generated considerable data with respect to integrase mutations conferring resistance to this class of compounds. *In vitro* resistance selection studies with the first series of diketo integrase inhibitors (i) identified mutations in the integrase gene leading to resistance, and (ii) suggested that accumulation of resistance mutations in the integrase gene during selection reduced viral replication[102]. *In vitro* passage experiments with L-708,906 selected for resistant viruses with the following mutation patterns in the integrase gene: T66I+S153Y and T66I+M154I[103] or T66I+S153Y+N155S[102]. Fikkert, et al. also identified integrase mutations T66I, L74M and S230R selected under L-708,906 selection pressure[105]. Another diketo acid, L-870,812, selected the N155H integrase mutation as the major drug resistance mutation in rhesus macaques after a month of exposure to the compound[105]. The diketo acid S-1360 selected T66I, L74M, Q146K, Q148K, I151L, and N155S as major integrase mutations, while other changes, including A128T, E138K, S153A, K160D, V165I, and V201I, were detected as mixtures along with the wild-type amino acids[106]. A naphthyridine carboxamide (L-870,810) sequentially selected mutations V72I, F121Y, T125K, and V151I over a period of nine months[108]. Recent additional *in vitro* selection studies with the integrase inhibitor L-870,810 resulted in the selection of L74M, E92Q, and S230N mutations[107]. A 1H-benzylindole analog (CHI/1043) has been linked to integrase mutations T66I and Q146K[108], while resistance to GSK-364735 (a compound in development by Shionogi and GlaxoSmithKline) has been associated with two different integrase resistance patterns (F121Y and Q148R) along with several additional mutations (T66I+E92Q, D64N+D116N, E138K, P145S, and Q148K)[109].

Phase II and III clinical trials involving highly HAART-experienced patients, some of whom subsequently experienced virologic failure on either raltegravir- or elvitegravir-based antiretroviral regimens, and several small clinical cohort studies of raltegravir have recently provided the most clinically relevant data on the *in vivo* resistance patterns selected by these antiretroviral drugs. Here we summarize the resistance patterns identified thus far for these two HIV-1 integrase inhibitors.

### *Resistance to raltegravir*

In independent dose-escalation experiments *in vitro*, raltegravir selected Q148K, E138A, and G140A mutations, or T66A, Q95K, and Y143C/R mutations in

HIV-$1_{IIIB}$ integrase[110]. The combination of the Q148K, E138A, and G140A mutations caused several hundred-fold reduced susceptibility to raltegravir and cross-resistance to elvitegravir. The Y143R integrase mutation caused > 10-fold reduced susceptibility to raltegravir, but had little effect on elvitegravir susceptibility. Selection experiments *in vitro* using a high multiplicity of infection (MOI) of HIV-$1_{HXB-2}$ and 100 nM of raltegravir resulted in breakthrough viruses carrying the N155H integrase mutation. The N155H mutation caused > 10-fold reduced susceptibility to raltegravir and elvitegravir. Eight independent *in vitro* resistance selection experiments with raltegravir (at 3 µM concentration) and HIV-$1_{IIIB}$ resulted in the emergence of the Q148R, E138K, and G140A integrase mutations in most cases[111].

Data on raltegravir resistance patterns *in vivo* has been reported in both antiretroviral-naive and experienced subjects. In a phase II study (Protocol 005) of raltegravir in ARV treatment-experienced subjects, 35/38 virologic failure subjects developed mutations in the integrase gene. Most subjects developed either the Q148H/R/K (n = 20) or N155H (n = 14) integrase mutations, either alone or combined with other integrase mutations; one subject also developed the Y143R integrase mutation, conferring 10- to 40-fold reduction in susceptibility to raltegravir[112]. Other integrase mutations detected that developed along with Q148H/R/K included L74M, E138A/K, and G140S. The most common pattern of mutations that developed was Q148H+G140S (13/35 subjects) and was shown to be associated with significant reductions in raltegravir susceptibility. Other integrase mutations that developed along with N155H included L74M, Y143H, V151I, G163K, D232N, E92Q, G163R, and T97A. Longitudinal analyses of Protocol 005 (virologic failure) demonstrated independent evolution of multiple integrase inhibitor-resistant viral quasispecies in patients failing raltegravir, with evidence of genotypic switching among integrase inhibitor resistance mutations in many subjects[113]. In particular, the initial development of an N155H or N155/Q148 pattern of integrase resistance mutations often evolved towards genotypes containing the Q148H/R/K mutations. Other subjects developed N155H or Q148R/H/K integrase mutations and maintained these patterns or added more integrase mutations. A minority of subjects either developed Y143C/R/H integrase mutations and switched to Q148H/R/K integrase mutations, or developed the N155H integrase mutation and switched to Y143C/R/H mutations. At the earliest time-points > 400 copies/ml at which integrase inhibitor genotypic resistance was detected, the majority of virologic failure subjects already had two or more integrase mutations.

Similar patterns of integrase resistance mutations were observed in the two phase III BENCHMRK studies of raltegravir in ARV treatment-experienced subjects[91]. Through week 48, integrase genotypes were obtained on 94/462 subjects treated with raltegravir, of which 81 had protocol-defined virologic rebound (> 400 copies/ml). Among these 94 patients, 64 developed one or more integrase mutations known to confer resistance to raltegravir, five developed amino acid changes in integrase of unknown significance, and 25 had no change in their integrase genotype relative to baseline. The Q148H/R/K integrase mutations were observed in 26 patients, N155H occurred in 38 patients, and Y143C/R mutations occurred in 10 patients. Other integrase inhibitor resistance mutations observed to develop included E92Q and L74M; several other integrase mutations of unknown significance were observed in some patients. Clonal analyses of baseline and virologic failure samples from the BENCHMRK studies (n = 69 subjects analyzed) confirmed that mutations at integrase codons Q148 and N155 developed on separate viral genomes, as did E92Q and Q148R/H/K mutations[114]. Integrase mutations G140S/A and E92Q were linked with Q148 and N155 mutants, respectively. Mutations E92Q plus N155H had increased resistance to raltegravir, but reduced replication capacity relative to either mutation alone. Mutation G140S had differential effects on raltegravir susceptibility when added to different Q148 integrase mutants, causing increased resistance to raltegravir when combined with Q148H/R mutations, but decreased resistance when combined with Q148K. Both E138K and G140S/A integrase mutations had differential effects on replication capacity, depending on the Q148 mutant background. Integrase replication capacity was generally reduced in the majority of subjects in whom integrase mutations developed, relative to their baseline samples.

Several small clinical cohort studies have also described integrase resistance data in ARV treatment-experienced subjects with virologic failure on raltegravir-containing regimens. The patterns of integrase resistance observed in these studies closely resembled those observed in raltegravir phase II/III studies, including Q148H+G140S/A, N155H, Y143C/R/H, E92Q, T97A, E138K, I151V, and D232N mutations[115-118]. In a phase II study of raltegravir in treatment-naive subjects (Protocol 004), 2/5 virologic failures on raltegravir

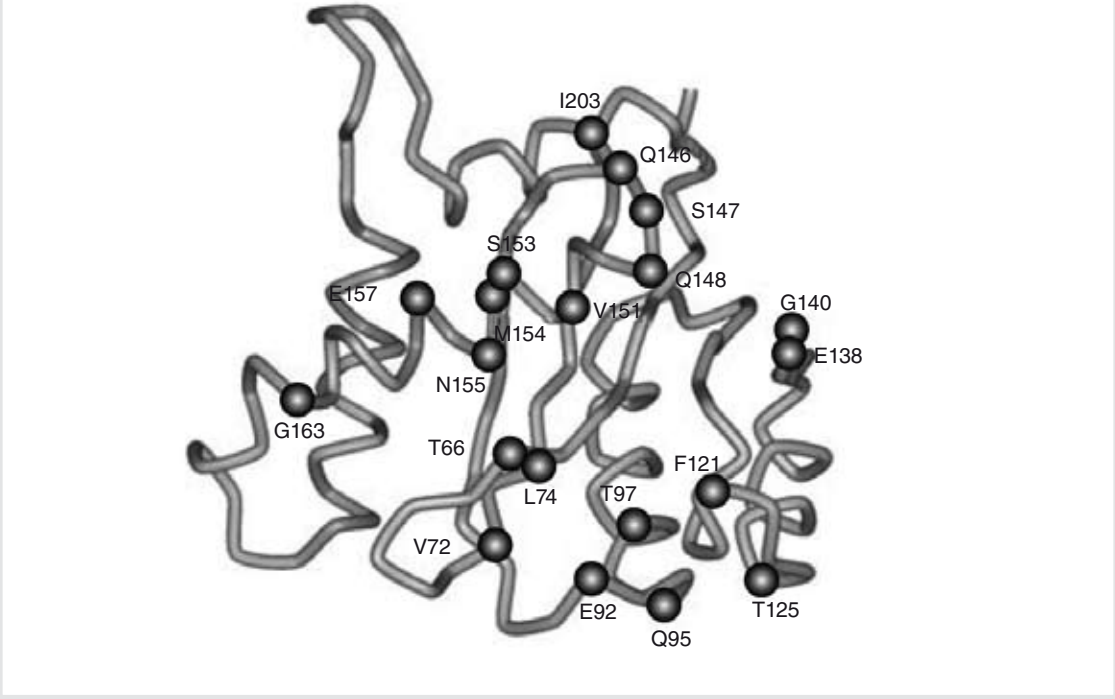



**Figure 4.** *Amino acid substitutions associated with resistance to integrase inhibitors in the integrase core crystal structure[145]. Mutations selected during in vitro passages with integrase inhibitors, or selected in vivo after therapy with raltegravir or elvitegravir, are indicated (amino acids H51, Y143, and S230 are not depicted due to imperfections on the HIV-1 integrase crystal structure). Numbering correspond to HIV-1$_{HXB2}$.*

combined with tenofovir/lamivudine developed integrase mutations, including N155H, V151I, G163R/G and D232D/N, all of whom were dosed with raltegravir 100 or 200 mg twice daily[119].

Based on these studies, moderate to high-level resistance to raltegravir seems to be associated with two major mutation "pathways" in the integrase gene[120,121] (Fig. 4). The N155 pathway involves histidine (or less commonly serine) at position 155, along with several secondary mutations including L74M, E92Q, T97A, V151L, and G163R. A second pathway seems to be related to primary mutations at codon 148 (i.e. Q148K/R/H) and linked to secondary mutations such as E138K and G140S/A. Two minor pathways associated with amino acid substitutions E92Q and Y143C/H/R have also been identified[112,104,122]. Mutations L74M, E92Q, T97A, I203M, and S230R have been associated to Y143C/H/R, some of which are also common to the N155H pathway. Interestingly, in the absence of Y143C/H/R, Q148K/R/H, or N155H, the E92Q mutation was selected simultaneously with T66A and subsequently followed by L74I[123]. Mutation E92Q alone was also observed in clones from patients failing raltegravir in a minority of patients from the BENCHMRK studies[114].

Mutations associated with the Q148 pathway seem to confer higher resistance than the N155 pathway (25- to 40-fold vs. 10- to- 15-fold, respectively) in the presence of different secondary mutations[112]. However, they are also associated with significantly reduced viral replication capacity in single-cycle assays, and reduced relative fitness compared to wild-type in competitive relative fitness assays[114,124] (see below). Of the different combinations of mutations, viruses carrying G140S+Q148H seem to be the most resistant to raltegravir[112,125], and strongly impair two of the three major steps of HIV-1 integration into the host genome: HIV-1 3' processing and strand-transfer reactions[122].

## *Resistance to elvitegravir*

Similar to raltegravir, *in vitro* studies designed to select elvitegravir-resistant viruses have identified several

mutations in the integrase gene that confer resistance. Shimura, et al. demonstrated that amino acid substitutions T66I and E92Q, located in the active site of the enzyme, mainly contributed to elvitegravir resistance[126] (Fig. 4). However, continued propagation of the viruses following the selection of these two primary mutations resulted in the selection of several different secondary mutations, which enhanced the resistance to elvitegravir up to > 1,000-fold, including Q95K, E138K, Q146P, and S147G for T66I, and H51Y, S147G, and E157Q for E92Q[126]. Another study showed that viruses carrying mutations T66I, T66I+S153Y, or T66I+M154I were resistant to elvitegravir[127]. The T66I integrase mutation was also observed in two independent dose-escalation selection experiments with elvitegravir and HIV-1 $III_B$ *in vitro*, conducted by Gilead Sciences[128]. In these experiments, the T66I mutation selected by elvitegravir was then combined with either F121Y or S153Y or R263K integrase mutations. Site-directed mutant HIV-1 carrying the T66I mutation showed approximately 15-fold reduced susceptibility to elvitegravir; addition of the F121Y or S153Y or R263K mutations further decreased elvitegravir susceptibility to 34-, 37- and 94-fold, respectively, relative to the wild-type virus. The T66I/A/K mutations were also selected in dose-escalation experiments *in vitro* with elvitegravir performed by GSK, along with V72A, E92Q/V, P145S, Q146L/S, and Q148K/R integrase mutations. The T124A and A128T integrase mutations, which may be natural integrase polymorphisms, also developed[129].

In high MOI breakthrough experiments, the Q148R integrase mutation appeared in HIV-$1_{HXB2}$ selected with 25 nM elvitegravir, whereas T66A, V72I, and N155S integrase mutations emerged under selection with 100 nM elvitegravir[110]. Eight independent selection experiments *in vitro* using HIV-$1_{IIIB}$ and elvitegravir at 3 μM concentration resulted in the emergence of multiple integrase mutation patterns, including T66I alone, E92Q alone, E92Q+T66A, T66I+R20K+L74M+S230R, T66I+Q148R+A128T+E138K+S230R, Q148R+E138K, and Q148Q/R+E92E/Q+E138E/K[111]. Viral pools containing these mutations showed evidence of reduced susceptibility to elvitegravir and raltegravir. Therefore, based on *in vitro* studies, elvitegravir can select several integrase inhibitor resistance mutations, including H51Y, T66I/A/K, V72A, L74M, E92Q, Q95K, F121Y, E138K, P145S, Q146L/P/S, S147G, Q148R/K, S153Y/F, N155S, E157Q, S230R, and R263K. Among these, Q148 and N155 represent integrase codons at which primary integrase inhibitor resistance mutations to raltegravir also occur. Therefore, elvitegravir and raltegravir may be cross-resistant with one another, depending on the integrase mutational path selected.

Recent reports from phase II clinical studies of elvitegravir identified integrase mutations in viruses obtained from patients failing elvitegravir/r 125 mg therapy. E92Q, Q148(R/K/H) and N155H were among the most common integrase mutations observed and occurred with similar frequency[130]. Other known integrase inhibitor resistance-associated mutations observed included H51Y, T66I/A/K, V72I, E138K, G140S/C, S147G, E157Q, and S230R. A novel integrase mutation, L68V/I, was observed to be associated only with the E92Q mutation and acted as a secondary mutation to increase resistance to both elvitegravir and raltegravir when added to E92Q. As for raltegravir, multiple independent viral quasispecies carrying integrase inhibitor resistance mutations were observed to evolve in patients failing elvitegravir, many of which carried patterns of integrase mutations predicted to cause cross-resistance to raltegravir, including mutations at Q148 and N155 integrase codons. Moreover, evidence of mutual exclusion among integrase mutations, including E92Q and Q148R, was observed in clones from patients failing elvitegravir, similar to what has been described for raltegravir. This suggests that there are functionally important biochemical incompatibilities among integrase mutations, or that certain combinations of mutations provide no added resistance benefit. Additional data and a more complete picture of the *in vivo* resistance patterns to elvitegravir should be available after the analysis of upcoming phase III clinical trials.

## *Cross-resistance among HIV-1 integrase inhibitors*

Several studies have shown the presence of significant genotypic and phenotypic cross-resistance between raltegravir and elvitegravir, including mutations E92Q, Q148R/K/H, and N155H[112,130] (Weber and Quiñones-Mateu, unpublished results), suggesting that a common mechanism is involved in resistance and potential cross-resistance to both integrase inhibitors. *In vitro* studies have shown that different combinations of mutations in the integrase gene confer cross-resistance not only to raltegravir and elvitegravir, but also to novel integrase inhibitors still in development. For example, mutations T66I+Q95K+Q146P+S147G, E92Q+S147G, and V72I+F121Y+T125K+V151I were associated with high-level resistance to elvitegravir and L-870,810[126];

the combination of E92Q+S147G also mediated low-level resistance (approximately 7-fold) to raltegravir. Similar results were obtained by Ren, et al. where viruses harboring mutations F121Y, F121Y+T125K, V72I+F121Y+T125K, or V72I+F121Y+T125K+V151I were resistant to raltegravir, elvitegravir, L-870,810, L-731,988, and pyridone A[127] (Fig. 4). Finally, a virus selected *in vitro* with mutations L74M+E92Q+S230N was shown to be resistant to L-870,810 and elvitegravir and had low-level reduced susceptibility to raltegravir[107]. Further studies will be necessary to understand the relevance of these combinations of mutations and potential cross-resistance to integrase inhibitors in the clinical setting. In particular, the question of what are the clinically relevant phenotypic cutoffs for integrase inhibitors remains to be determined.

## *Resistance to integrase inhibitors in non-B HIV-1 and HIV-2*

As described above, multiple mutations in several positions of the integrase gene have been associated with resistance to the two principal integrase inhibitors, raltegravir and elvitegravir. Natural polymorphisms in these positions may potentially have important implications in the treatment of individuals with integrase inhibitors. Although the catalytic triad of the HIV-1 integrase (i.e. positions D64, D116 and E152, and the HHCC motif at the N-terminal of the enzyme) is highly conserved in most viruses[131,132], few studies have analyzed the sequence variability on integrase genes among viruses from different HIV-1 subtypes and groups. Lataillade, et al. showed that natural polymorphisms were present in 21 of the 42 positions currently associated with resistance to integrase inhibitors, notably E138K, Q148H, V151I, M154I, and I203M[133]. However, no polymorphisms have been observed in positions associated with other integrase inhibitor resistance mutations, i.e. T66, E92, G140, S147, or N155[131]. Leoz, et al. obtained similar results after analyzing 96 sequences from isolates or clinical samples from individuals infected with HIV-1 group O; natural mutations were observed in several positions that have been linked with resistance to integrase inhibitors[134]. Interestingly, six group O sequences had the E157Q mutation, which has been associated with resistance to raltegravir[122] and elvitegravir, probably as a secondary resistance mutation[126,128]. Finally, relatively high variability was shown in HIV-2 subtype A and B viruses (28 and 30%, respectively), but none at codons associated with resistance to raltegravir or elvitegravir[132]. It is evident that with the increasing use of integrase inhibitors worldwide, more studies will be needed to monitor the impact of these natural polymorphisms in integrase inhibitor-based antiretroviral regimens.

## *Replicative fitness of viruses resistant to integrase inhibitors*

The effects of resistance mutations to HIV-1 integrase inhibitors on viral replicative fitness have yet to be fully described. Initial *in vitro* selection of viruses resistant to the diketo acid L-708,906 showed that triple mutant variants (i.e. T66I+L74M+S230R) have impaired 3' processing and strand-transfer activities[135]. This effect in enzymatic activity was associated with a decrease in the replication kinetics when compared to the wild-type HIV-1 strain. Similar results were obtained during *in vitro* passage of HIV-$1_{IIIB}$ in the presence of increasing concentrations of the diketo analog S-1360[136]. A total of nine amino acid substitutions were identified in the catalytic domain of the integrase, including T66I and L74M, which have been associated with resistance to L-708,906 and more recently to raltegravir and/or elvitegravir (see above for details). Reduced replication fitness was observed for all mutant strains compared to the wild-type strain[136]. The N155S mutation, which confers cross-resistance to both diketo acids and naphthyridine carboxamide, has been associated with a 70% reduction in replicative capacity as measured in single-cycle assays[104]. Finally, resistance against another Merck compound, L-870,810, was accompanied by a reduction in viral replication kinetics[107].

As described above, resistance to raltegravir and other drugs in the pipeline, such as elvitegravir and S/GSK364735, may be driven by single mutations, suggesting that a decrease in viral replicative fitness may be associated with the selection of primary mutations associated with resistance to integrase inhibitors. Moreover, and similar to the phenomenon observed with other drug classes such as PI and RTI[137,138], the selection and order of addition of secondary mutations to primary integrase inhibitor mutations can be associated with a rebound in viral fitness[109,112,130,139]. Interestingly, one of the most fit raltegravir-resistant viruses is also one showing the highest level of resistance, namely virus harboring integrase mutations G140S+Q148H[112]. Several studies have indicated that

the combination of the G140S+Q148H mutations confers significantly increased viral replication relative to the Q148R/H/K mutations alone. Thus, the virus achieves two goals by making this particular combination of integrase mutations: high-level resistance combined with partial compensation for viral replication defects resulting from primary integrase inhibitor resistance mutations (Q148H). In contrast, other combinations of integrase mutations, such as N155H+E92Q, result in increased resistance to raltegravir (and elvitegravir), but the resulting virus has reduced replication capacity relative to either wild-type or the individual single mutants[124].

In the case of elvitegravir, viruses resistant to this integrase inhibitor showed a significant reduction in their replicative capacity relative to both wild-type virus and other integrase inhibitor-resistant variants selected by L-870,810[126,130]. Recent studies have confirmed that single primary integrase resistance mutations such as E92Q, Q148R/H/K, and N155H can cause reductions in viral replication capacity in single-cycle assays or reduced relative fitness in competitive-fitness assays[114,124]. Some secondary integrase mutations, such E138K and G140S, can partially compensate for reduced viral replication of primary integrase inhibitor resistance mutations; however, these compensatory effects can be dependent on the particular primary integrase inhibitor resistance mutation pattern present[114,124].

To date, the clinical impact of reduced viral fitness of amino acid substitutions in the integrase gene alone or, more importantly, when combined within a background of PI, NRTI, and/or NNRTI associated resistance mutations is not completely understood. As in the case of recently developed antiretroviral drugs, the success of phase III clinical trials and further approval by the FDA will likely lead to new studies and a better understanding of the effects of integrase inhibitor resistance on HIV-1 replicative fitness.

## Perspectives: integrase inhibitors as microbicides?

Approximately 33 million people worldwide are infected with HIV-1 and some three million new HIV-1 infections are estimated to occur every year (http://data.unaids.org/pub/EPISlides/2007/2007_epiupdate_en.pdf). Despite the fact that HIV-1 was identified and isolated a quarter of a century ago, an effective vaccine against HIV-1 remains elusive. Furthermore, as discussed in this review, integration of the viral genome into the chromosomal DNA of the HIV-infected human host is an essential part of the viral lifecycle. As a result, this means that many quiescent $CD4^+$ cells in an infected host carry latent virus, which may subsequently go on to initiate productive viral replication. Eradication of all HIV-1-infected $CD4^+$ T-cells in the immune system (i.e. "HIV cure") has therefore been estimated to require periods ranging from less than seven years to the entire lifetime of an infected individual, even if absolutely perfect antiretroviral suppression could be maintained and depending on the point in the infection at which antiretroviral therapy was initiated[140,141]. In an era where the use of HAART for HIV-1 treatment is widespread and expanding, many individuals are therefore carrying strains of HIV-1 with antiretroviral drug resistance to one or more classes of existing drugs. Thus, prevention of transmission of HIV-1, and particularly of drug-resistant HIV-1, is an urgent priority and in the absence of an effective vaccine, use of antiretroviral drugs as microbicides to prevent transmission may represent an effective public health measure to slow the spread of the epidemic.

Microbicides, and in particular vaginal microbicides, may be a particularly effective intervention in slowing the epidemic, as 50% of all new transmissions worldwide occur in women, particularly in countries with very high prevalence rates of HIV-1[142,143]. New classes of drugs, such as integrase inhibitors, which are both highly potent and to which the majority of infected patients in the world are naive, may be particularly effective in this respect, as strains resistant to existing classes of drugs such as NRTI, NNRTI and PI remain fully susceptible to integrase inhibitors. Furthermore, even though integrase inhibitors act intracellularly after entry of the viral particle into an infected cell, inhibition of this critical viral step represents a "last line of defense" against establishment of a successful infection by HIV-1. Integration is a highly regulated process, and failure to integrate the viral genome due to blockage by an integrase inhibitor leads to non-productive intracellular viral DNA intermediates, 1-LTR and 2-LTR circles.

Initial studies *in vitro* with the naphthyridine carboxamide integrase inhibitor L-870,812 have demonstrated the capacity of this compound, when present at 1-10 μM concentrations, to block cell-free and cell-associated infection by $HIV\text{-}1_{BaL}$ in co-cultures of monocyte-derived dendritic cells and $CD4^+$ T-cells, the primary target cells in sexual transmission of

HIV-1[144]. Furthermore, at the concentrations tested, no acute or delayed toxic effects of the compound on the primary cells tested were observed. Further studies of their safety, stability, formulation, and efficacy are needed to establish the use integrase inhibitors as microbicides for the prevention of HIV-1 transmission. The highly potent nature of integrase inhibitors and their good safety profile in clinical trials to date, combined with the absolute necessity of HIV-1 to successfully integrate for productive viral replication, means that integrase inhibitors could offer many advantages for both treatment of established HIV-1 infection and further prevention of HIV-1 transmission as microbicides.

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