Published in final edited form as:

Curr Opin Chem Biol. 2013 June ; 17(3): 339–345. doi:10.1016/j.cbpa.2013.04.010.

Allosteric inhibition of HIV-1 integrase activity

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Abstract

HIV-1 integrase is an important therapeutic target in the fight against HIV/AIDS. Integrase strand transfer inhibitors (INSTIs), which target the enzyme active site, have witnessed clinical success over the past 5 years, but the generation of drug resistance poses challenges to INSTI-based therapies moving forward. Integrase is a dynamic protein, and its ordered multimerization is critical to enzyme activity. The integrase tetramer, bound to viral DNA, interacts with host LEDGF/p75 protein to tether integration to active genes. Allosteric integrase inhibitors (ALLINIs) that compete with LEDGF/p75 for binding to integrase disrupt integrase assembly with viral DNA and allosterically inhibit enzyme function. ALLINIs display steep dose response curves and synergize with INSTIs *ex vivo*, highlighting this novel inhibitor class for clinical development.

Introduction

AIDS results from the systemic replication of the pathogenic lentivirus human immunodeficiency virus type 1 (HIV-1), which in the vast majority of cases if unchecked destroys the host immune system. Great strides have been made over the past 15 years to control HIV-1 through the administration of highly active antiretroviral therapy (HAART), a combinatorial approach that in most cases targets the activities of the viral reverse transcriptase and protease enzymes (reviewed in [1]). Despite this remarkable success, significant numbers of new infections are mediated by drug-resistant viral strains [2]. With questionable hope for a vaccine in the foreseeable future, development of derivative drugs that work against currently exploited targets in the face of resistance, and the development of novel compounds that inhibit previously unexploited aspects of the viral lifecycle, are the best lines of attack against HIV/AIDS [1].

The integration of the double-stranded viral DNA reverse transcript into cellular chromatin, which is mediated by the viral integrase (IN) enzyme, is essential to HIV-1 replication. IN catalyzes two spatially and temporally distinct chemical reactions, 3' processing and DNA strand transfer, in the context of the retroviral preintegration complex (PIC). IN binds the U3 and U5 ends of viral DNA to form the stable synaptic complex (SSC) or intasome in the cell cytoplasm (Figure 1). IN then processes the viral DNA 3' ends, yielding reactive CA_{OH}-3'

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termini that serve as substrates for the subsequent DNA strand transfer reaction. After nuclear entry and locating a suitable chromatin acceptor site, IN uses the CA_{OH} ends to cut the chromosome in staggered fashion, which at the same time joins the viral DNA ends to target DNA 5'-phosphates. The resulting integration intermediate is repaired by host cell enzymes to yield the integrated provirus (reviewed in [3]) (Figure 1).

The licensing in 2007 of the first IN inhibitor, raltegravir (RAL), which targets the enzyme active site and inhibits DNA strand transfer activity (Figure 1), increased the breadth of the HAART armamentarium [4]. Expectedly, RAL-resistance emerges in treated patients, and, moreover, these mutations in large part confer resistance to the second licensed IN strand transfer inhibitor (INSTI), elvitegravir. Dolutegravir, a second-generation INSTI in late phase clinical trials, retains activity against many of the RAL-resistance viruses, indicating it may prove utile against first generation drug resistant strains [5]. Alternatively, small molecules that inhibit IN activity by engaging the enzyme at sites that are distinct from the active site should retain potency in the face of INSTI resistance mutations [6–8]. This review discusses recent advances in the targeting of the IN-IN dimerization interface with small molecules that have proven allosteric inhibitory activity.

Retroviruses target chromatin in distinct manners during integration. HIV-1 prefers active genes within transcriptionally active gene-dense regions of chromosomes (reviewed in [9]). Research over the past several years has clarified roles for virus-host interactions in this aspect of HIV-1 biology. The propensity to integrate along the bodies of active genes is in large part dictated by an interaction between IN and lens epithelium-derived growth factor (LEDGF)/p75 [9]. LEDGF/p75 functions as a bimodal tether during integration: elements within its N-terminal region, which include a PWWP domain, nuclear localization signal, and two copies of an AT-hook DNA binding motif, confer constitutive binding to chromatin, whereas a C-terminally located IN-binding domain (IBD) binds lentiviral IN proteins (Figure 2a). LEDGF/p75 is one of six members of the hepatoma-derived growth factor (HDGF) related-protein (HRP) family; whereas all members contain an N-terminal PWWP domain, HRP2 is the only other member to harbor an IBD [10] (Figure 2a). LEDGF/p75 and HRP2 each stimulate recombinant HIV-1 IN activity *in vitro* [10], and HRP2 can also tether HIV-1 integration to active genes, though this has been observed only in the backdrop of LEDGF/p75 depletion [11,12]. The targeting of gene dense regions of chromosomes is primarily dictated by the viral capsid protein and host factors important for PIC nuclear import, including transportin 3, nucleoporin 358 (NUP358), and NUP153 [13,14].

IN dynamics and HIV-1 intasome assembly

HIV-1 IN is comprised of three independently folded protein domains, the N-terminal domain, catalytic core domain (CCD), and C-terminal domain, and IN multimerization is essential for catalytic function (reviewed in [15]) (Figure 1 and 2b). A tetramer of IN catalyzes the pairwise insertion of both viral DNA ends; although a dimer of IN can suffice to process a single viral DNA end *in vitro*, it seems likely that 3' processing is also catalyzed by the IN tetramer during virus infection [16] (reviewed in [3]). A series of elegant intasome crystal structures based on prototype foamy virus, a member of the spumavirus genus of *Retroviridae*, has elucidated the structural basis for retroviral DNA integration [16–18]; the basic PFV intasome architecture moreover seems applicable to HIV-1 [19–21]. The intasome contains a tetramer of IN, with two inner subunits directly engaging the viral DNA ends (Figure 3a and [16,17]). Each inner subunit interacts with its outer partner through an extensive CCD-CCD interface, and LEDGF/p75 binds lentiviral IN proteins at a cleft formed at this interface [22] (Figure 3a, b). Small molecules that compete with LEDGF/p75 for binding to the CCD-CCD pocket are allosteric IN inhibitors (ALLINIs; Figure 3c).

HIV-1 IN is a highly dynamic protein and its ordered, temporal assembly with cognate DNA and cellular LEDGF/p75 co-factor is crucial for catalytic function [20,23,24]. Although LEDGF/p75 and its IBD can enhance the formation of tetrameric IN in the absence of viral DNA [24,25], LEDGF/p75-induced tetramers are unavailable for SSC formation [20]. The IBD counteracted stimulation of HIV-1 IN activity by LEDGF/p75 *in vitro* [10], and over-expression of the IBD in target cells accordingly inhibited HIV-1 infection at the integration step [26,27]. Such results first suggested that peptides derived from the IBD could display antiviral activity. The IBD is a right-handed helical bundle composed of two helix-hairpin-helix repeats, with each showing similarity to HEAT (Huntingtin/elongation factor 3/subunit A of protein phosphatase 2A/yeast PI3K Tor1)/ARM (Armadillo) repeats [28]. Co-crystallization of the IBD with the HIV-1 IN CCD revealed that LEDGF/p75 amino acids at the tips of the hairpins intimately engage IN at the CCD-CCD dimer interface [22]. LEDGF/p75 residue Asp366 contacts one IN subunit via bidentate H-bonds to the primary amino groups of IN residues Glu170 and His171, while LEDGF/p75 residue Ile365 nestles into a hydrophobic pocket that is comprised primarily of residues (Leu102, Ala128, Trp132) from the other IN subunit (Figure 3b). ALLINIs recapitulate the polar/non-polar chemistries of these amino acid interactions (Figure 3c).

ALLINIs that engage IN at the LEDGF/p75 binding site

Initial hints that the molecular details of the IBD-CCD interaction could form the basis of novel antiviral compounds came from studies of peptides based on the LEDGF/p75 IBD sequence [29,30]. Peptides that harbored the critical Ile365 and Asp366 contact residues enhanced tetramerization of IN in the absence of viral DNA [29] and inhibited IN-viral DNA [29,30] and IN-LEDGF/p75 binding [30]. Peptide-induced multimerization of IN in the absence of viral DNA [29] hinted at the mechanistic basis for allosteric inhibition of IN activity by small molecules that engage IN at the LEDGF/p75 binding site.

Two different approaches led to the identification of quinoline-based compounds that upon development yielded ALLINIs that inhibit LEDGF/p75-IN binding and IN catalytic activity *in vitro*, and HIV-1 replication in cell culture [31,32]. Christ and colleagues [31] identified LEDGIN1 from a commercial library of 200,000 compounds using an *in silico* pharmacophore that was primarily based on the IBD-CCD co-crystal structure, whereas Fenwick and colleagues [32] identified BI-A using a high throughput screen for inhibitors of IN 3' processing activity. Though neither compound inhibited HIV-1 replication, both displayed promising *in vitro* activities: LEDGIN1 at 100 μ M inhibited the LEDGF/p75-IN interaction by 36%, whereas 9 μ M BI-A inhibited 50% of IN 3' processing activity. Compounds that inhibit HIV-1 replication with effective concentration 50% (EC₅₀) values in the 10 nM-100 nM range were subsequently developed from both leads [32–34] (Figure 4).

Surprisingly, despite very close structural similarity of the quinolone-based compounds, initial reports suggested two distinct mechanisms of action. BI-1001, which was derived from BI-A, preferentially inhibited IN 3' processing activity [32] whereas CX0516 or LEDGIN6, which was developed from LEDGIN1, selectively impaired IN-LEDGF/p75 binding [31]. We therefore evaluated BI-1001 and LEDGIN6 in parallel using a series of assays, which revealed that similar concentrations of each compound (4–10 μ M LEDGIN6; 1–2 μ M BI-1001) inhibited LEDGF/p75-IN binding and LEDGF/p75-independent IN 3' processing and DNA strand transfer activities *in vitro* [35]. Because the inhibitors co-crystallize at the LEDGF/p75 binding site [31,33,35], which is distal from the enzyme active site (Figure 3), they are *bona fide* allosteric IN inhibitors or ALLINIs. ALLINIs induce HIV-1 IN multimerization in the absence of viral DNA [33–35], and accordingly inhibit the formation of the SSC *in vitro* [33,35]. The sequencing of viral DNAs indicates that the 3'

processing step of HIV-1 integration is indeed inhibited during acute HIV-1 infection [33]. The key pharmacophore carboxylic acid mimics the bidentate H-bonding pattern of LEDGF/p75 residue Asp366 with the IN polypeptide backbone (Figure 3b, c; [31,33,35]). The presence of a *tert*-butoxy moiety moreover tracks with ALLINI potency (Figure 4).

ALLINIs display steep antiviral dose response curves *ex vivo*, revealing that IN behaves as a multivalent drug target under these conditions [35]. This contrasts with the INSTIs, which display slopes of ~1 due to the inhibition of a single intasome per infectious virus particle [17,36]. Based on available data, the inhibition of two integration-related functions, SSC assembly (and hence IN catalysis) and the association of the PIC with LEDGF/p75, has been proposed to account for the multimodal action of ALLINIs [33–35]. The contribution of the IN-LEDGF/p75 interaction to ALLINI potency is however unclear: compound potency is increased when LEDGF/p75 [37] or both HRP2 and LEDGF/p75 [11] are depleted from target cells, revealing that the host factors can compete with the small molecules for IN binding during acute HIV-1 infection. However, if the protein interactions were the crucial drug targets, we would have expected ALLINI potency to decrease, not increase, upon removal of the host binding partners from the cell [11].

ALLINIs inhibit infection by a wide range of HIV-1 subtypes [34] but are ineffective against HIV-2 [31], indicating that antiviral activity may be restricted to the HIV-1/SIV_{cpz} group of viruses. Selection of ALLINI-resistant HIV-1 strains *ex vivo* has been reported by numerous groups, with the lion share of resistance mutations (Y99H, L102F, A124D, A128T, H171T) mapping to the LEDGF/p75 binding pocket on IN [31–33]. ALLINIs importantly retain activity against virus strains resistant to other classes of HIV-1 inhibitors, including INSTIs [31], and the different inhibitor classes are accordingly active against ALLINI-resistant viruses [33,34]. ALLINI and INSTI activities are additive *ex vivo* [34], indicating that the two different classes of IN inhibitors could be effective in combination therapy. A potential roadblock to the clinical development of ALLINIs is their apparent low genetic barrier to resistance.

Allosteric inhibition of IN activity by compounds that engage distinct CCD binding sites

ALLINIs that engage the LEDGF/p75 binding site inhibit IN activity by enhancing the formation of IN multimers, which precludes SSC assembly (Figure 1). Molecules that engage the CCD-CCD interface at positions other than the LEDGF/p75 binding site and either enhance [38] or disrupt [39,40] the formation of IN dimers have also been described, but in neither case has their development advanced to the same stage as the LEDGF/p75 binding site ALLINIs.

A structural analog of chicoric acid that engages a pocket formed by the CCD-CCD dimerization interface adjacent to the LEDGF/p75 binding site inhibits IN activity in the low μ M range and, akin to the LEDGF/p75 binding site compounds, restricts the exchange of IN monomers in solution and enhances the formation of higher-order IN multimers [38]. In line with its engagement of the CCD at a site that is distinct from the site of LEDGF/p75 binding, the compound did not inhibit the LEDGF/p75-IN interaction.

The CCD-CCD interaction is primarily dictated by contacts mediated through alpha helices 1 and 5 within the domain. Peptides INH1 and INH5, derived from alpha helices 1 and 5, respectively, inhibited IN dimerization and catalysis *in vitro*; INH5 was the more potent of the two peptides, displaying IC₅₀s of ~85 nM and 60 nM in 3' processing and DNA strand transfer assays, respectively [39]. Virtual screening more recently identified a series of

compounds that predictably engage the CCD nearby alpha helices 1 and 5 [40]; one such compound disrupted IN dimerization in the mid μM -range.

Conclusions

The results of several studies highlight the sensitive nature of HIV-1 IN dynamics for the development of novel anti-HIV compounds. In theory, molecules that either disrupt or enhance the formation of the CCD-CCD dimer could serve as ALLINIs, and the best characterized of these are the series of quinolone-based molecules that engage the interface at the LEDGF/p75 binding site (Figures 3 and 4). The field has highlighted two integration-associated functions, SSC assembly and the SSC-LEDGF/p75 interaction, that potentially contribute to the steep dose response curves of these inhibitors. The gain in ALLINI strength observed in LEDGF/p75 knockout cells indicates that the host factor and ALLINIs indeed compete for binding to the same IN pocket, though raise questions as to the relevance of the IN-LEDGF/p75 (or IN-HRP2) interaction in determining compound potency [11]. Virus produced in the presence of ALLINIs is non-infectious [34], and recent findings indicate that compound-induced multimerization of IN during viral egress underlies the multimode mechanism of drug action [41]. The resultant particles are defective for particle core maturation, reverse transcription, and integration [41]. The perturbation of multiple replication steps, combined with their ability to work with INSTIs *ex vivo* [34], highlights the ALLINIs for clinical development.

Acknowledgments

The authors acknowledge funding support from the US National Institutes of Health (AI039394 to A.E.; AI097044 to J.J.K.; AI062520 and AI081581 to M.K.; GM103368 to A.E. and M.K.) and thank Lei Feng and Kellie A. Jurado for helpful comments.

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- of outstanding interest

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Highlights

- Integrase (IN), an essential HIV-1 enzyme, is an important therapeutic target.
- Allosteric IN inhibitors (ALLINIs) are an emerging class of anti-HIV drug.
- ALLINIs promote IN dimerization and block IN-viral DNA assembly and IN catalysis.
- ALLINIs display steep dose response curve slopes *ex vivo*.
- ALLINIs synergize with strand transfer inhibitors that target the IN active site.

Figure 1.

IN activities and integration reaction pathway. HIV-1 IN exists in various forms, from monomer to higher-order multimers, in solution. IN binding to the ends of the viral DNA (open triangle, upstream U3 end of viral DNA; black triangle, downstream U5 end) results in the formation of the stable synaptic complex (SSC). IN processes the 3' ends of the viral DNA adjacent to conserved CA sequences (short vertical arrows) in the cell cytoplasm. The PIC engages chromosomal target DNA in the nucleus, followed by IN-mediated DNA strand transfer activity that joins the recessed viral DNA $\text{CA}_{\text{OH}}\text{-}3'$ ends to the 5'-phosphates of a staggered double-stranded DNA cut (marked by short vertical arrows in chromosomal DNA). The resulting integration intermediate, with unjoined viral DNA 5' ends, is repaired by host cell machinery to yield the integrated provirus flanked by the sequence duplication of the staggered DNA cut. Investigational ALLINIs promote IN multimerization and thereby inhibit SSC formation (and both 3' processing and DNA strand transfer activities); clinical INSTIs preferentially inhibit DNA strand transfer activity.

Figure 2.

Protein domain organizations. (a) Primary domain organizations of HRP2 and LEDGF/p75. Numbers represent positions of amino acid boundaries. HR, homology region; CR, charged region. (b) HIV-1 IN domains. Numbers represent amino acid position boundaries and lengths of interdomain linkers and C-terminal tail (from [15]). Letters within boxes are amino acid residues conserved among retroviral IN proteins; the Asp and Glu residues of the D,D-35-E active site motif are highlighted in red. NTD, N-terminal domain; CCD, catalytic core domain; CTD, C-terminal domain. Colored boxes in panels (a) and (b) demarcate important interaction domains (see Figure 3).

Figure 3. HIV-1 intasome model and crystal structure of BI-1001 bound to the HIV-1 IN CCD dimer. (a) Model of the HIV-1 intasome from reference [20]. The inner subunits are colored cyan and green, whereas the outer subunits are gray and blue. The LEDGF/p75 IBD is in magenta, and the viral DNA strands are in orange. The red spheres mark the positions of inner subunit active site residues Asp64, Asp116, and Glu152. (b) Close-up view of the LEDGF/p75-IBD interaction from reference [22] (protein database code 2B4J). H-bonding between LEDGF/p75 IBD residue Asp366 and the backbone amides of IN residues Glu170 and His171 are shown by dashed lines. (c) BI-1001 binding to the HIV-1 IN CCD dimer interface from reference [35] (protein database code 4DMN). H-bonding between the BI-1001 carboxylic acid and backbone amides of Glu170 and His171 are shown by dashed lines.

Figure 4.

Representative ALLINI structures. The BI (Boehringer Ingelheim) series of compounds was referred to by Fenwick and colleagues [32] as non-catalytic site IN inhibitors (NCINIs). GS (Gilead Sciences) licensed the NCINI program from BI in October 2011; the GS series of compounds was reported in [33]. One of the more promising compounds from the BI/GS series, BI-224436 (not shown), was slated in 2011 for a phase I clinical trial, but the study was withdrawn prior to enrollment. The LEDGIN (for LEDGF/p75-IN inhibitor) series of compounds was developed by Christ and colleagues [31,34]; CX0516 and CX05045 are also referred to as LEDGIN6 and LEDGIN7, respectively [31]. We collectively refer to these compounds as ALLINIs to highlight their multimodal mechanism of action. Numbers below the compounds (ND, not determined) are EC₅₀ values for inhibition of HIV-1 replication in C8166 (BI compounds) and MT-4 (GS and LEDGIN compounds) cells; CX0516, B1-1001, and BID EC₅₀ values in SupT1 cells were 12.2 μM , 5.8 μM , and 0.09 μM , respectively [35,41].