CHAPTER THREE

HIV Integrase Inhibitors: 20-Year Landmark and Challenges

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Contents

Abstract

Since the discovery of HIV as the cause for AIDS 30 years ago, major progress has been made, including the discovery of drugs that now control the disease. Here, we review the integrase (IN) inhibitors from the discovery of the first compounds 20 years ago to the approval of two highly effective IN strand transfer inhibitors (INSTIs), raltegravir (Isentress) and elvitegravir (Stribild), and the promising clinical activity of dolutegravir. After summarizing the molecular mechanism of action of the INSTIs as interfacial inhibitors, we discuss the remaining challenges. Those include: overcoming resistance to clinical INSTIs, long-term safety of INSTIs, cost of therapy, place of the INSTIs in prophylactic treatments, and the development of new classes of inhibitors (the LEDGINs) targeting IN outside its catalytic site. We also discuss the role of chromatin and host DNA repair factor for the completion of integration.

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ABBREVIATIONS

3′-P 3′-processing
CCD catalytic core domain
CTD C-terminal domain
DTG dolutegravir
EVG elvitegravir
HAART highly active antiretroviral therapy
IN integrase
INSTI integrase stand transfer inhibitor
LEDGIN LEDGF/p75-integrase inhibitor
NNRTI nonnucleoside reverse transcriptase inhibitor
NRTI nucleoside reverse transcriptase inhibitor
NTD N-terminal domain
PIC preintegration complex
RAL raltegravir
RT reverse transcriptase
ST strand transfer

1. INTRODUCTION

Human history has been marked by pandemics resulting from parasitic (malaria), bacterial (plague), and viral (flu) infections. The Center for Disease Control and prevention (CDC) alerted about acquired immunodeficiency syndrome (AIDS) in 1981, and the etiological agent, human immunodeficiency virus (HIV), was isolated in 1983 (Barre-Sinoussi et al., 1983). Azidothymidine (AZT) was approved shortly after on March 1987 as the first nucleoside reverse transcriptase (RT) inhibitor (NRTI) for the treatment of AIDS (Yarchoan & Broder, 1987). As resistance to AZT quickly emerged by mutations of its target RT (Larder & Kemp, 1989), it became clear that resistance could be minimized by combining drugs together in a highly active antiretroviral therapy (HAART) to raise the genetic barrier (Larder, Darby, & Richman, 1989). The first bitherapy was made publicly available in 1997 [AZT in combination with lamivudine (3TC)], 10 years after AZT approval. The number of drugs approved increased at an unprecedented speed (Fig. 3.1), making available new classes of molecules (nonnucleoside RT inhibitors, NNRTI) as well as drugs acting on new viral targets [protease (PR) inhibitors, PI; entry/fusion inhibitors; integrase (IN) strand transfer (ST) inhibitors, INSTIs]. Today, with 27 drugs available (Table 3.1), the therapeutic choices are multiple and have changed the prognosis of HIV

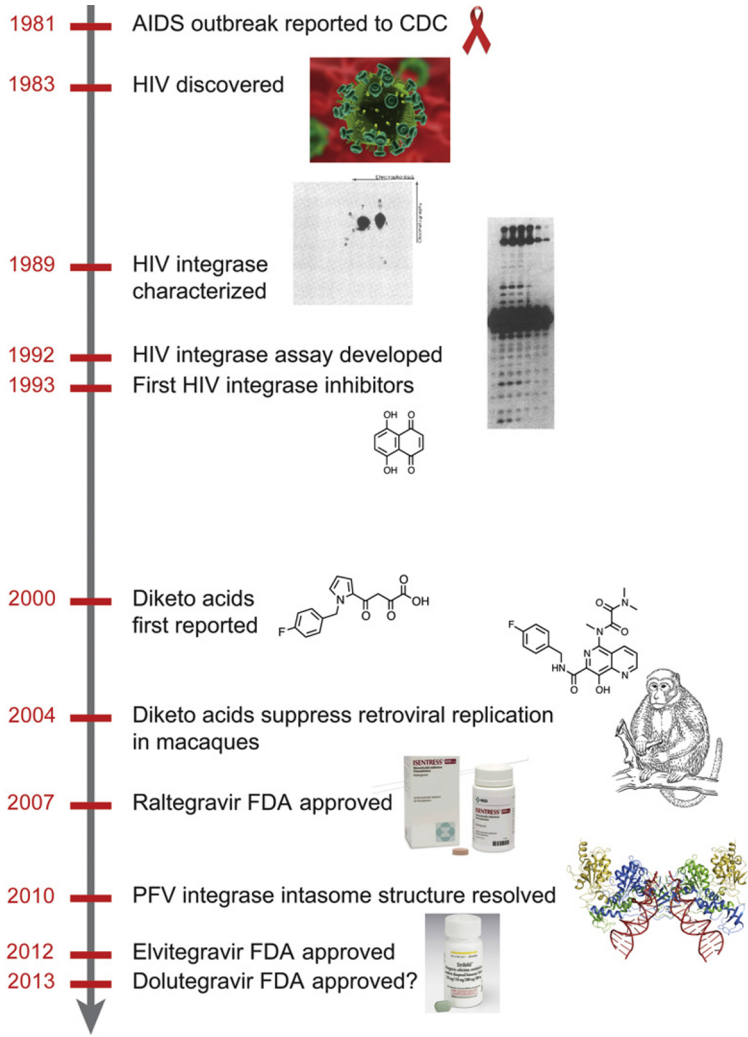

Figure 3.1 Timeline focusing on HIV-1 integrase inhibitor discovery.

infections in the developed world. Recently, single daily pills with high efficacy and practical convenience are becoming the norm (Marchand, 2012). Providing access to the drugs in low- and middle-income countries remains a major challenge (globally, only 54% of people eligible to therapy actually are under treatment; Vazquez, 1996).

Table 3.1 List of the FDA-approved anti-HIV drugs and formulations (www.fda.org)

Approval date	Brand name	Class	Category	In combination with		Manufacturer
2012	Stribild	Multitarget combination	INI + NRTI	Elvitegravir Cobicistat Emtricitabine Tenofovir disoproxil fumarate	QD	Gilead Sciences
2011	Edurant		NNRTI	Rilpivirine	QD	Tibotec Therapeutics
2011	Complera	Multiclass combination	NRTI + NNRTI	Rilpivirine Emtricitabine Tenofovir disoproxil fumarate	QD	Gilead Sciences
2008	Intelence		NNRTI	Etravirine	BID	Tibotec Therapeutics
2007	Selzentry		CCR5 antagonist	Maraviroc	BID[a]	Pfizer
2007	Isentress		INI	Raltegravir	BID	Merck & Co.
2006	Atripla	Multiclass combination	NRTI + NNRTI	Efavirenz Emtricitabine Tenofovir disoproxil fumarate	QD	Bristol-Myers Squibb Gilead Sciences
2006	Prezista		PI	Darunavir	QD[b]	Tibotec Therapeutics

2005	Aptivus		PI	Tripranavir (TPV)	BID[b]	Boehringer Ingelheim
2004	Epzicom	Combination	NRTI	Abacavir Lamivudine	QD[a]	GlaxoSmithKline
2004	Truvada	Combination	NRTI	Emtricitabine Tenofovir disoproxil fumarate	QD	Gilead Sciences
2003	Emtriva		NRTI	Emtricitabine (FTC)	QD	Gilead Sciences
2003	Lexiva		PI	Fosamprenavir calcium (FOS-APV)	BID[b]	GlaxoSmithKline
2003	Fuzeon		Fusion inhibitor	Enfuvirtide (T-20)	BID[c]	Hoffmann-La Roche Trimeris
2003	Reyataz		PI	Atazanavir sulfate (ATV)	QD[b]	Bristol–Myers Squibb
2001	Viread		NRTI	Tenofovir disoproxil fumarate	QD	Gilead Sciences
2000	Trizivir	Combination	NRTI	Abacavir Lamivudine Zidovudine	BID	GlaxoSmithKline
2000	Kaletra	combination	PI	Lopinavir Ritonavir	BID	Abbott Laboratories

Continued

Table 3.1 List of the FDA-approved anti-HIV drugs and formulations (www.fda.org)—cont'd

Approval date	Brand name	Class	Category	In combination with		Manufacturer
1999	Agenerase		PI	Amprenavir (APV)	BID[a]	GlaxoSmithKline
1998	Ziagen		NRTI	Abacavir sulfate (ABC)	BID[a]	GlaxoSmithKline
1998	Sustiva		NNRTI	Efavirenz (EFV)	QD[a]	Bristol–Myers Squibb
1997	Combivir		NRTI	Lamivudine Zidovudine	BID	GlaxoSmithKline
1997	Rescriptor		NNRTI	Delavirdine (DLV)	TID[a]	Pfizer
1997	Fortovase[d]		PI	Saquinavir	TID or BID[b]	Hoffmann-La Roche
1997	Viracept		PI	Nelfinavir mesylate (NFV)	TID or BID	Agouron Pharmaceuticals
1996	Viramune[e]		NNRTI	Nevirapine (NVP)	QD[a]	Boehringer Ingelheim
1996	Crixivan		PI	Indinavir (IDV)	QID	Merck & Co.
1996	Norvir		PI	Ritonavir (RTV)	BID[a]	Abbott Laboratories
1995	Epivir		NRTI	Lamivudine (3TC)	QD[a] or BID[a]	GlaxoSmithKline

1995	Invirase	PI	Saquinavir mesylate (SQV)	BID[b]	Hoffmann-La Roche
1994	Zerit	NRTI	Stavudine (d4T)	BID	Bristol–Myers Squibb
1992	Hivid[d]	NRTI	Zalcitabine (ddC)	QID[a]	Hoffmann-La Roche
1991	Videx[e]	NRTI	Didanosine (ddI)	QD or BID	Bristol–Myers Squibb
1987	Retrovir	NRTI	Zidovudine (ZDV, AZT)	BID[a] or TID[a]	GlaxoSmithKline

QD, *quaque die* (once a day); BID, *bis in die* (twice daily); TID, *ter in die* (three times a day); QID, *quater in die* (four times a day); INI, integrase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, nonnucleoside reverse transcriptase inhibitor; PI, protease inhibitor.
[a]Must be given in combination with other antiretroviral medications.
[b]Must be administered with ritonavir.
[c]Injection.
[d]No longer marketed.
[e]Newer formulation available (i.e., extended release or enteric coated).

2. REQUIREMENT OF IN FOR HIV REPLICATION

HIV is a retrovirus coding for structural (*env*), nonstructural (*gag*-pol), and accessory proteins (Nef, Rev, Tat, Vif, Vpr, and Vpu; Cullen, 1991). Its replication requires both viral and cellular enzymes. IN is one of the three viral enzymes encoded by the *POL* gene, together with RT and PR. To replicate, the virus first attaches to cells harboring the membrane receptor CD4 (Fig. 3.2), explaining its selectivity for dendritic cells, macrophages, and T-lymphocytes. Interactions with a coreceptor (either CCR5 or CXCR4) initiate conformational changes of the viral gp120–gp41 complex, enabling membrane fusion and release of the viral core into the cytoplasm. Viral RNA is reverse-transcribed into a double-stranded viral DNA (vDNA) copy (Fig. 3.2), which is assembled into a large nucleoprotein complex with IN bound to its ends (preintegration complex, PIC; Bukrinsky et al., 1993).

IN carries out vDNA integration following two consecutive steps: 3′-processing (3′-P) in the cytoplasm and ST in the nucleus. For 3′-P, IN processes vDNA by cleaving its 3′-end immediately after a conserved CA dinucleotide, thereby releasing a GT dinucleotide from each long terminal repeats (LTRs) 3′-ends (Figs. 3.2 and 3.3). The PIC then translocates to the nucleus, where IN binds to the cellular target DNA. ST is carried out by IN tetramers (see Section 3), allowing the concerted integration of both extremities of the linear vDNA, five bases from each other on opposite strands, producing a 5-nucleotide sequence that is repeated at each side of the fully integrated proviral DNA (Fig. 3.3). Thus, to complete the integration process, ST products need to be processed and fully sealed with the host genome. This "repair" step requires removal of the two mispaired nucleotides at the 5′-ends of the vDNA and gap filling (Fig. 3.3 and Section 5.5). Once repaired, transcription, translation, and maturation of the different viral components lead to the assembly of new particles budding out of the cell. Of note, a small but consistent fraction of PIC (around 1%) can undergo different processes after nuclear import (Jurriaans, de Ronde, Dekker, Goudsmit, & Cornelissen, 1992). Those include end-joining, homologous recombination, or autointegration (IN dependent) and produce circular forms of vDNA (1-LTR and 2-LTR circles, Fig. 3.3; Craigie & Bushman, 2012). Inhibition of IN markedly increases the proportion of those forms, raising the question of potential episomal expression or DNA reservoirs for later integration (Cara & Klotman, 2006).

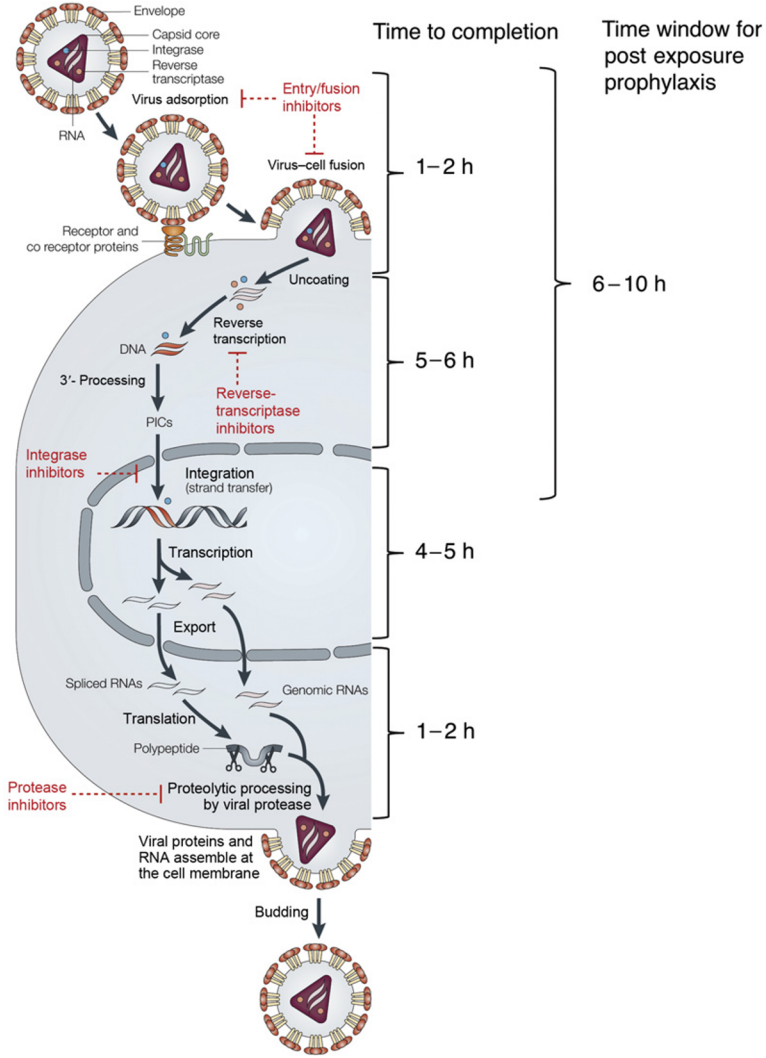

Figure 3.2 HIV-1 replication cycle. Clinically targeted steps are highlighted in red/dark gray. Time frame for postinfection prophylaxis with INSTIs is indicated on the right. *Adapted from Metifiot, Marchand, Maddali, and Pommier (2010) and Pommier et al. (2005).*

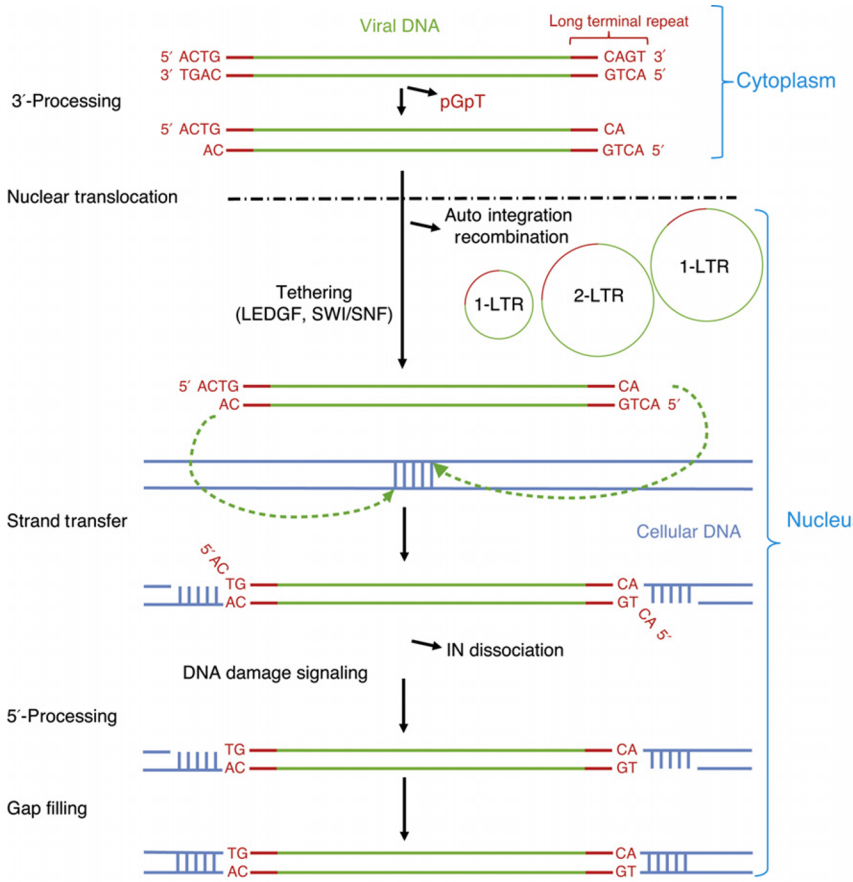

Figure 3.3 Schematic steps for integration.

3. IN STRUCTURE

HIV-1 IN is a 288 amino acid long (32 kDa) polypeptide belonging to a family of proteins including RNase H, Ruv C, transposases, and other retroviral INs (Engelman & Cherepanov, 2012). IN contains three domains. The N-terminal domain (NTD, amino acids 1–49) comprises a zinc-binding motif, HHCC, important for oligomerization. IN residues 50–212 correspond to the catalytic core domain (CCD), including a D-D$_{35}$-X motif ($D_{64}D_{116}E_{152}$) conserved among the IN superfamily. The C-terminal domain (CTD, amino acids 213–288) contains an SH3-like

domain involved in nonsequence-specific DNA binding. Overall, the three domains of IN bind DNA as an oligomer.

IN, in its active form, the intasome, is a tetramer with two active sites (Fig. 3.4), each using two magnesium ions coordinated by a triad of conserved acidic residues (the DDE motif). To date, no crystal structure of the full-length HIV-1 IN has been resolved. However, studies involving the three isolated domains demonstrated that all three form homodimers in solution. Resolution of the combinations NTD–CCD and CCD–CTD structures in addition to electron microscopy experiments have permitted the modeling of a full-length structure(Chen et al., 2000; Ren, Gao, Bushman, & Yeager, 2007; Wang, Ling, Yang, & Craigie, 2001). However, in those structures lacking the vDNA, a flexible loop surrounding the active site (amino acids 140–149) is poorly resolved, voiding the possibility of rational drug design.

After 20 years, the first crystal structure of a full-length IN in complex with its DNA substrate (intasome) has been solved (Fig. 3.4; Hare, Gupta, Valkov, Engelman, & Cherepanov, 2010). This intasome is from the prototype foamy virus (PFV), a Spumavirus quite divergent from its HIV-1 counterpart outside of the active site (Yin & Craigie, 2010). Nonetheless, this first insight in the complete architecture of the intasome enabled the generation of more accurate homology models for HIV-1 IN (Johnson, Metifiot, Pommier, & Hughes, 2012; Krishnan et al., 2010). These models include the vDNA and allow docking and rational design of inhibitors in the active site of HIV-1 IN.

4. THE INSTIs

The first IN inhibitors (INI) were reported 20 years ago (Fesen, Kohn, Leteurtre, & Pommier, 1993), only 10 years after the discovery of HIV as the agent responsible for AIDS, and approximately 15 years before the Food and Drug Administration (FDA)'s approval of raltegravir. These inhibitors belonged to various pharmacological categories including DNA binding drugs, antimalarial agents, naphthoquinones, and flavones (Fig. 3.1). Of note, a common feature of these compounds was a metal chelating catechol group (Fesen et al., 1993). The next milestone was reached 7 years later with the report by Hazuda and coworkers from the Merck research laboratories of the diketo acids as the first INSTIs to inhibit viral replication at nanomolar concentrations in infected cells (Hazuda, Iwamoto, & Wenning, 2009; Hazuda et al., 2000). Four years later, the same group provided the proof

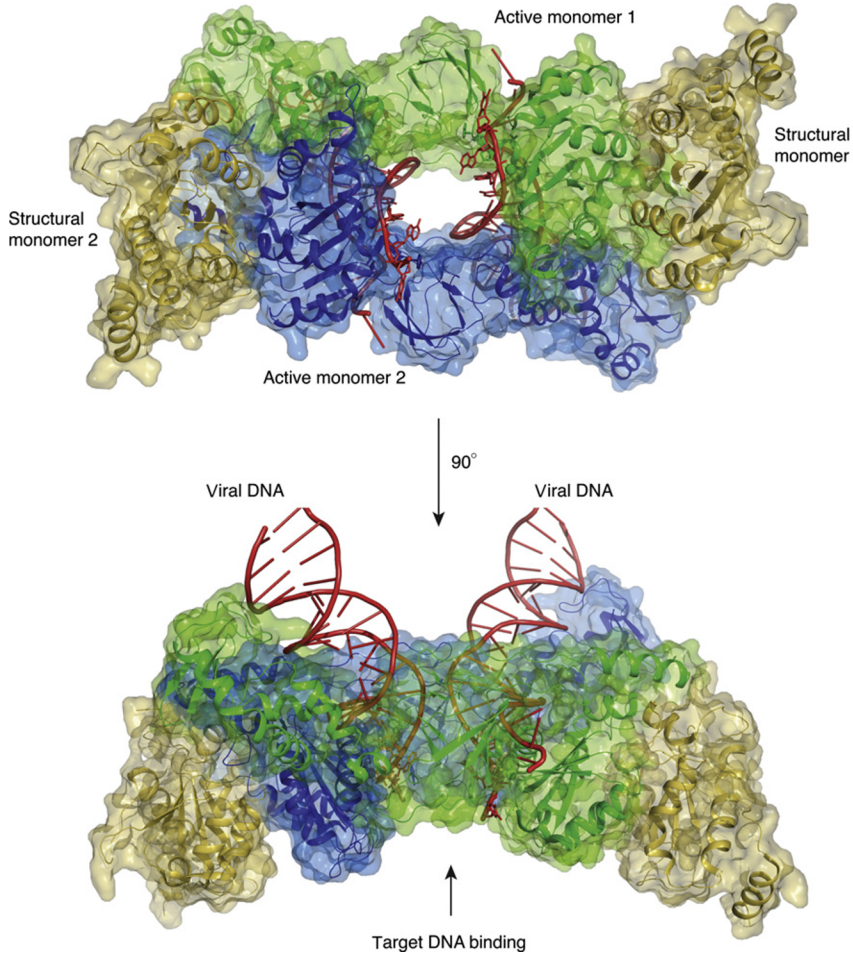

Figure 3.4 Structure of the intasome. The tetrameric PFV IN structures containing the viral DNA are derived from the PDB ID 4E71 (Hare et al., EMBO 2012). Proteins are shown in surface and cartoon, and the magnesium cofactors as spheres. Structural outer subunits (noncatalytically active) are represented in yellow/light gray and inner active subunits are represented in blue/dark gray and green/medium gray, respectively. The viral DNA is shown in red cartoon (dark gray) and the last four bases on the cleaved strand are presented as sticks. The bottom representation corresponds to a 90° rotation of the top view.

of principle that INSTIs can efficiently suppress viral replication in rhesus macaques (Hazuda et al., 2004). Raltegravir (RAL, Isentress®, Merck & Co.) was approved in 2007 by the FDA as the first INSTI for heavily treated AIDS patients with multidrug resistance. Two years later, RAL was approved for naïve patients in first-line therapy, and since, the use of RAL has been extended to pediatric patients and patients with co-hepatitis B or C infections. RAL is well tolerated but in a small fraction of patients selects for resistance mutations in the IN gene. Resistance to RAL arises from three major mutation sites at positions 155 (N155H), 143 (Y143R), and 148 (G140S/Q148H double mutant). RAL is used in a twice-daily administration, which does not favor treatment adherence. Therefore, new INSTIs have been developed to overcome RAL resistance with a once-daily administration.

Elvitegravir (EVG) was approved as the second INSTI for the treatment of HIV/AIDS in August 2012. It is formulated as the "quad pill" (Stribild®, Gilead Sciences) together with the pharmaco-enhancer cobicistat, and the two NRTIs emtricitabine and tenofovir disoproxil fumarate (Marchand, 2012). Cobicistat, by boosting the blood concentration of EVG, allows EVG to be administered at 150 mg once daily with a safety and efficacy comparable to raltegravir 400 mg twice daily. Because EVG does not structurally interact with the HIV-1 IN residue Y143, it alleviates RAL resistance resulting from mutation at this position (Table 3.2; Metifiot, Vandegraaff, et al., 2011). Unfortunately, RAL resistance mutation at position 148 (double mutant G140S/Q148H) remains highly cross-resistant to EVG (Table 3.2; Metifiot, Vandegraaff, et al., 2011).

Dolutegravir (DTG) is in advanced stage clinical development as a once-daily single formulation in the 572-Trii pill (Shionogi-ViiV Healthcare, LLC). Like the "quad pill," 572-Trii combines both IN and RT inhibitors. DTG (50 mg, unboosted) is packaged with two NRTIs, lamivudine and abacavir (van Lunzen et al., 2012). DTG (formerly S/GSK1349572) has a higher genetic barrier than RAL and EVG with respect to selecting for drug-resistance mutations in IN (Hare et al., 2011; van Lunzen et al., 2012). The double mutant G140S/Q148H is less than 4-fold resistant to DTG, whereas it is resistant to RAL by several 100-fold (Table 3.2). Hightower et al. recently used RAL, EVG, and DTG to measure each drug's residence time within the IN active site. Surprisingly, EVG seems to be the weakest binder in the context of the WT enzyme, and the reported residence time of the drugs are about 3, 9, and 71 h for EVG, RAL, and DTG, respectively (Hightower et al., 2011). More importantly, mutations involved in INSTIs resistance increase the dissociation rate of the clinical drugs, and only DTG retains a long

Table 3.2 Clinically relevant integrase mutations conferring resistance to INSTIs used in the treatment of HIV/AIDS

Resistance mutations	Raltegravir	Elvitegravir	Dolutegravir	References
N155H	30–50	50–60	<4	Hare et al. (2011), Metifiot, Vandegraaff, et al. (2011), and Quashie et al. (2012)
Y143R	42–50	2	<4	Hare et al. (2011), Metifiot, Johnson, et al. (2011), Metifiot, Vandegraaff, et al. (2011), Quashie et al. (2012), and Wainberg et al. (2012)
G140S/ Q148H	400–770	2200	<4	Hare et al. (2011), Metifiot, Johnson, et al. (2011), Metifiot, Vandegraaff, et al. (2011), Quashie et al. (2012), and Wainberg et al. (2012)
E138K/ Q148K	>150	>150	≈20	Fransen et al. (2009), Johnson et al. (2006), Kobayashi et al. (2011), and Quashie et al. (2012)
Q148R/ N155H	>140	>300	≈10	Kobayashi et al. (2011)
G118R	2–10	<2	2–10	Hare et al. (2011), Quashie et al. (2012), and Wainberg et al. (2012)
R263K	1–2	6	11	Margot et al. (2012) and Quashie et al. (2012)
R263K/ H51Y	NR	NR	15	Mesplede et al. (2012)

Antiviral resistance is expressed as fold-change compared to wild type. NR, not reported. Note the importance of Q148 mutations for INSTI resistance.

retention time (>5 h), which correlates with its better activity against those mutants. However, the improved resistance profile of DTG is not yet fully understood (Johnson et al., 2012). Clinical resistance to DTG has not yet been reported, but IN mutations G118R, E138K, Q148K, R263K, and H51Y have been selected in infected cells cultured in the presence of DTG (Table 3.2; Quashie et al., 2012; Wainberg, Mesplede, & Quashie, 2012).

RAL, EVG, and DTG have all been cocrystallized in the PFV IN active site (Hare et al., 2010, 2011). They all share the same interfacial inhibition mechanism. Interfacial inhibitors are drugs that target macromolecular complexes by binding at the interface of at least two macromolecular components, thereby stabilizing a conformation intermediate that interferes with the dynamic activity of the macromolecular complex (Fig. 3.5; Pommier, Johnson, & Marchand, 2005; Pommier & Marchand, 2012). In the case of INSTIs, the macromolecular complex is the intasome consisting of the catalytic site of one IN polypeptide, the vDNA, and the two catalytic magnesium cations (Fig. 3.5D). All the INSTIs establish molecular contacts with each single component of the intasome complex (Metifiot, Marchand, Maddali, & Pommier, 2010). For all INSTIs, three oxygen or nitrogen atoms coordinate the two magnesium ions in the IN catalytic triad residues (DDE motif; Fig. 3.5). At the same time, the INSTIs also bind by stacking their halobenzyl group with the 3′-penultimate cytosine of the vDNA, while the terminal adenosine of the vDNA is displaced from the active site (Fig. 3.5).

5. CHALLENGES

This section is a selected list of questions, challenges, and possible answers regarding IN biology and drug targeting (see Table 3.3).

5.1. Resistance to INSTIs

The HIV genome is highly dynamic due to low fidelity of RT, and RAL resistance can evolve readily in the clinic (Malet et al., 2008; Wainberg et al., 2012). Drug-resistance mutations are determined both by drug selection and viral fitness (Fransen et al., 2009; Hazuda, 2010; Metifiot, Marchand, et al., 2010; Quercia, Dam, Perez-Bercoff, & Clavel, 2009; Wainberg et al., 2012). However, viral fitness is impacted by deleterious mutations in key catalytic residues and in residues involved in IN structural stability. Thus, emergence of resistant viruses depends on the selective advantage of such mutants, defined by the resistance level and viral fitness. The probability of such deleterious mutations increases with the number of mutations, which explains why combination of INSTIs with RT inhibitors is likely the most effective way to impede the occurrence of drug-resistance mutations. Indeed, to overcome three different classes of drugs, HIV must undergo at least three independent mutations (at least one for each drug), not counting additional secondary mutations to compensate for lack of fitness

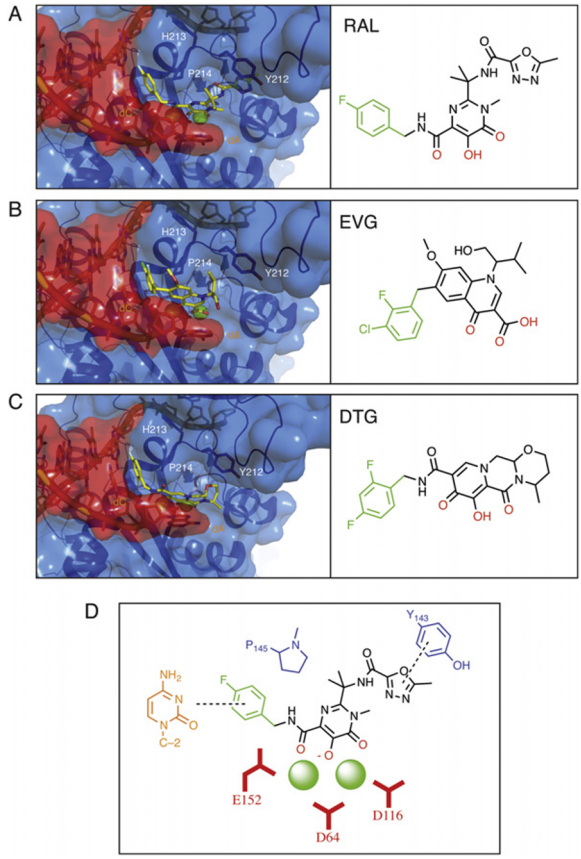

Figure 3.5 INSTIs interfacial binding to the intasome. (A–C, left panels) zoom-in view of the PFV IN active site containing metal, DNA, and drugs [pdb ID 3OYA (RAL, panel A), 3L2U (EVG, panel B), and 3S3M (DTG, panel C)]. (A–C, right panels) chemical structures of RAL, EVG, and DTG. INSTIs' main features are colored in red/dark gray and green/medium gray to highlight the chelating triad and the halobenzyl ring, respectively. (D) Scheme showing the interfacial inhibition mechanism of RAL. Π-stacking of the halobenzyl ring with the penultimate cytosine of the viral DNA and of the oxadiazole ring with the tyrosine residue of IN are indicated by dashed lines.

due to the primary drug-resistance mutations (for instance, the associated 140 mutations that rescue the deleterious effects of 148 mutations and confer full drug resistance; Fransen et al., 2009; Metifiot, Maddali, et al., 2010; Quercia et al., 2009). Treatment adherence is another key factor since the probability of mutations is directly linked to viral replication, which can resume if drug levels fall below therapeutic range. Compared to

Table 3.3 Challenges for the discovery and use of IN inhibitors

Challenges	Possible answers (new approaches)
Resistance to INSTIs	• Combination treatments • Treatment adherence: multidrug once-daily formulations • Novel INSTIs with different genetic barriers • Drugs targeting different regions of IN • Dual IN and RT inhibitors?
Long-term safety of INSTIs	• Follow cancer incidence and long-term side effects
Prophylaxis and cost	• INSTIs for pre- and postinfection prophylaxis • Microbicides • Regulatory agreements for price determination • Novel inhibitors (competition, nonprofit drug discovery such as FightAIDS@Home)
Novel IN inhibitors targeting different IN sites	• LEDGINs • Target IN dimerization • Discovery of IN cofactors and targeting their IN interface • Allosteric inhibitors of IN (Flexible loop site) • 3′-processing inhibitors with IN selectivity (RNase H)
Role of DDR and repair pathways for complete integration	• Model systems and techniques to elucidate the molecular DNA repair pathways and cellular responses following ST (5′-processing, gap filling, and provirus chromatinization) • New therapeutic opportunities?

raltegravir, both the recently approved EVG quad pill (Stribild®) and the DTG 572-Trii pill in late clinical trials are multidrug (IN + RT) once-daily pills, addressing the combination and treatment adherence issues, and therefore should reduce the risk of drug resistance.

Yet, when drug resistance due to IN mutations occurs, new drugs are necessary (Hazuda, 2010). EVG can overcome one of the RAL mutations (Y143R) but neither the G140S/Q148H nor the N155H mutations

(Metifiot, Vandegraaff, et al., 2011). Fortunately, DTG partially overcomes those mutations (Hare et al., 2011) and could therefore be used as salvage therapy in RAL- or EVG-resistant viruses. It is still unknown which resistance mutations will be seen in DTG-treated patients. None have been reported so far, and the genetic barrier for DTG appears higher than for RAL. The known DTG-resistance mutations have been generated in cell lines exposed for months to suboptimal concentrations of the drug (Table 3.2; Quashie et al., 2012). Clinical use of DTG will reveal whether and which IN mutations are clinically relevant. The next question will then be whether those mutations confer cross-resistance to RAL and ELG. In any case, it is clear additional INSTIs will be useful to overcome DTG-resistance (Agrawal et al., 2012; Metifiot et al., 2013; Taoda et al., 2012). It also appears that mutations at IN residue Q148 are a common determination of high levels of drug resistance (Table 3.2), suggesting that Q148 is a critical determinant of the IN catalytic site where the INSTIs bind (Johnson et al., 2007, 2006).

Another approach to overcome INSTI resistance is to develop drugs that target IN out of its active site (Table 3.3). LEDGINs, discussed in detail below are not cross-resistant with INSTIs and combination experiments demonstrate that LEDGINs and INSTIs act additively, suggesting a rationale for combining both INIs together (Christ & Debyser, 2013).

5.2. Long-term safety and tolerance for INSTIs

After 5 years of clinical use, it is clear that RAL (Isentress®) remains remarkably well tolerated. Rare side effects (less than 5% of patients) include insomnia, headache, nausea, and fatigue. One case of severe cerebral ataxia under RAL therapy has been reported (Reiss, Bailey, Pham, & Gallant, 2010). Because EVG is administered in the Quad pill (together with cobicistat, emtricitabine, tenofovir disoproxil fumarate) (Stribild®), it will be more difficult to determine EVG-specific long-term side effects. Yet, Stribild® is remarkably well tolerated. Diarrhea and nausea occur in less than 15% of patients and headaches and insomnia in less than 10% of patients.

In spite of the absence of a cellular equivalent of IN, human proteins structurally related to IN can be inhibited by some INSTIs at relatively high concentrations. This justifies testing new INSTIs against RAG1/2 recombinase, RNase H, or Metnase (Marchand et al., 2008; Melek et al., 2002; Williamson et al., 2012). Interfering with V(D)J recombination, which depends on RAG1/2 (Melek et al., 2002) could lead to immune disorders and opportunistic infections. Inhibition of Metnase

(Williamson et al., 2012), which is involved in DNA repair, topology, and stability (De Haro et al., 2010; Ponder et al., 2011; Wray et al., 2010), might produce neurotoxicity, immune disorders, and increase cancer risk. Another consideration is the accumulation of abortive unintegrated viral copies (2-LTR circles; see above) in infected cells treated with INSTIs. Such circular vDNA copies might insert into functionally important genomic sites, potentially giving rise to secondary malignancies or immune defects. Long-term follow-up studies will reveal the incidence of cancers and immune or neurological disorders in INSTIs-treated patients.

5.3. Economical considerations and prophylactic treatments

Cost is a major limitation for HIV therapies, especially in low-income countries. While the price of a single antiretroviral varies from a couple of hundred to tens of thousands of dollars per year (Hill, Cho, & Mrus, 2011), combination therapies are expensive ($30,000–40,000 per year). For RAL alone, the cost exceeds $10,000 per year. For Stribild®, the price tag is $28,500 per year. Low-cost generics have reduced the price of anti-HIV therapies by threefold (Venkatesh, Mayer, & Carpenter, 2012). Yet, generics remain a complex legal issue. It is questionable whether new INSTIs or other classes of INIs can be developed at low cost.

Preventing HIV infections with drugs is economically sound as the cost of treatments is offset by their short duration. RT inhibitors effectively prevent mother to child transmission. However, RT inhibitors are not optimum for postexposure prophylaxis unless they can be given immediately after exposure. Indeed, RT is completed within 2–4 h following viral entry. On the other hand, integration takes between 5 and 10 h (Hazuda et al., 2009; Fig. 3.2). Therefore, INSTIs should be advantageous compared to RT inhibitors in postexposure prophylaxis. INIs in combination with RT and PR inhibitors could also be used for local prophylaxis. In which case, cheaper drugs that do not fulfill oral bioavailability might be useful.

The use of antiretroviral agents as microbicides has been considered recently for INIs (Crucitti, Botta, & Di Santo, 2012; Terrazas-Aranda et al., 2008). However, formulation will be a key determinant for ease of use and efficacy over time. A main constraint in preexposure prophylaxis is the safety of and resistance to available drugs (Abraham & Gulick, 2012). Similar to HAART, it seems that microbicides should combine multiple drugs to prevent transmission of resistant viruses. Of note, S/GSK1265744 (ViiV Healthcare), which is now in clinical phase 2b as

an oral pill, has an apparent residence time of 30 h (Taoda et al., 2012). Recent conferences contained presentations that showed an impressive time of residence of 21–50 days by injection, which would be compatible with a one every 1–3 months administration. This approach could be advantageous in developing countries, where access to people at risk is limited. One could imagine a prophylactic dose for every person coming to be tested, protecting that individual for months to come.

5.4. Novel INIs targeting IN outside its catalytic site

5.4.1 The LEDGINs

Even in the most biochemical reductionist view, IN functions as a multimer (Fig. 3.4) with several protein–protein interfaces moving around each other to confer IN oligomeric flexibility and productive formation of the intasome. In a broader physiological view, IN functions with cofactors that regulate its activity. LEDGF/p75 was identified as an IN cofactor by coimmunoprecipitating overexpressed IN from human cells (Cherepanov et al., 2003). LEDGF/p75 is critical for HIV replication because it tethers IN to a host chromosome for integration, protects IN from proteasomal degradation, and stimulates its catalytic activity (reviewed in Christ & Debyser, 2013). A crystal structure of the LEDGF/p75 IN binding domain with a dimer of the IN catalytic core identified two regions of the IN dimer interface mediating the interaction: A128, W131, W132 and G168, E170, T174, M178 (Cherepanov, Ambrosio, Rahman, Ellenberger, & Engelman, 2005). This dimer interface region provides a defined binding pocket for LEDGF/p75 with multiple hydrophobic and hydrogen bond interactions favorable for drug binding.

The LEDGINs are small molecules rationally designed to bind to the LEDGF/p75 IN dimer interface binding pocket (reviewed in Christ & Debyser, 2013). Cocrystal structures confirmed that LEDGINs bind at the IN dimer interface in the LEDGF/p75 pocket (Christ et al., 2010). By stabilizing IN multimerization nonproductively and restricting IN oligomeric flexibility, LEDGINs inactivate allosterically (at a distance) the IN catalytic site (Christ & Debyser, 2013; Christ et al., 2012; Kessl et al., 2012; Tsiang et al., 2012). Thus, LEDGINs can be viewed as interfacial inhibitors (Pommier & Marchand, 2012; as they bind at the interface of 2 IN monomers) that act allosterically on the IN active site. They can also be viewed as competitive inhibitors with respect to LEDGF/p75. In contrast to INSTIs, LEDGINs are not selective for ST over 3′-P. They also must be

added prior to the DNA substrate to observe biochemical IN inhibition (Christ et al., 2012; Kessl et al., 2012; Tsiang et al., 2012). Several LEDGINs, derived from CX014442, have nanomolar antiviral potencies and are in advanced preclinical development (Christ & Debyser, 2013). Yet, the successful clinical development of LEDGINs will require optimization for bioavailability, pharmacokinetics, and toxicological studies. Moreover, LEDGINs will remain susceptible to drug resistance. *In vitro* selection of resistant viruses showed that single mutations in the IN LEDGF binding site, such as H99Y and A128T are sufficient to confer drug resistance (Christ et al., 2012, 2010). To limit the occurrence of IN resistance mutations, targeting LEDGF/p75 directly has recently been reported. Phage display screening led to the discovery of a cyclic peptide inhibitor of HIV replication (Desimmie et al., 2012).

5.4.2 Peptides targeting the LEDGF/p75 binding site

Short peptides targeting the LEDGF/p75 IN binding site have also been studied (Al-Mawsawi, Christ, Dayam, Debyser, & Neamati, 2008; Hayouka et al., 2012, 2007; Rhodes, Peat, Vandegraaff, Jeevarajah, Newman, et al., 2011). However, their potency remains inferior to LEDGINs and cellular delivery is even more challenging than for the LEDGINs. Peptides corresponding to IN dimerization interfaces (α5) or derived from interacting proteins (LEDGF, SNF5) were identified as potent inhibitors (Cherepanov, Devroe, Silver, & Engelman, 2004; Maroun et al., 2001; Yung et al., 2001).

5.4.3 Inhibitors targeting other cellular cofactors

IN binds other protein cofactors beside LEDGF/p75 (Cherepanov et al., 2003), including RT (Wu et al., 1999), INI1/hSNF5 (Cano & Kalpana, 2011; Kalpana, Marmon, Wang, Crabtree, & Goff, 1994), Rev (Rosenbluh et al., 2007), and Vpr (Bischerour et al., 2003; Fletcher et al., 1997). Based on the LEDGF/p75 example, defining such interactions might offer opportunities to discover inhibitors targeting these additional IN–protein interfaces by designing inhibitors mimicking the structural segments of the cofactor binding to IN. This approach recently led to the discovery of IN inhibitory peptides derived from RT (Armon-Omer et al., 2008) and Vpr (Gleenberg, Herschhorn, & Hizi, 2007; Suzuki, Maddali, et al., 2010; Suzuki, Urano, et al., 2010). In addition, inhibiting IN–cofactor interactions could influence other stages of viral replication, as evidenced by class II IN mutants, which are defective in viral assembly, particle

production, reverse transcription, or nuclear import, while retaining catalytic integration activity (Engelman, Englund, Orenstein, Martin, & Craigie, 1995; Leavitt, Robles, Alesandro, & Varmus, 1996; Li, Koh, & Engelman, 2012; Lu et al., 2004; Wiskerchen & Muesing, 1995). A recent study indicates that an acetylated form of IN could interact with transportin 3 and Nup358 (Allouch & Cereseto, 2011). Targeting IN at these cofactor binding sites will potentially lead to novel classes of integration inhibitors.

5.4.4 Allosteric inhibitors of IN

A novel inhibitor binding site has recently been reported outside the IN catalytic site. This new binding site accommodates a new series of INIs discovered using catalytic assays, surface plasmon resonance, and saturation transfer difference NMR to screen a fragment-based library (Rhodes, Peat, Vandegraaff, Jeevarajah, Le, et al., 2011). Cocrystallographic experiments have confirmed that these inhibitors bind in proximity to the IN flexible loop (residues 140–149) and contact the three IN residues: Q62, S147, and H183 (Rhodes, Peat, Vandegraaff, Jeevarajah, Le, et al., 2011). Further structural, binding, and mechanistic studies should provide more insight on this new binding pocket, which may represent an alternative to alleviate clinical resistance to INSTIs.

5.5. Chromatin and DNA repair cofactors completing integration

IN-mediated ST leaves two nicked DNA segments in a host chromosome, each with a 2-base 5′-vDNA mismatch and a 5-base gap in the host sequence at the junction of the viral and host DNAs (Fig. 3.3). Processing of these intermediates relies on cellular repair (Yoder & Bushman, 2000) and chromatin factors following disassembly of the intasome.

A parallel can be drawn between retroviral integration and Mu transposition (Mizuuchi & Craigie, 1986). Following integration into the bacterial chromosome, the transposome formed by MuA, which carries out a reaction similar to IN, needs to be disassembled by action of the host factor ClpX, which mediates MuA unfolding and destabilization to permit completion of Mu transposition (Burton & Baker, 2003). IN posttranslational modifications include phosphorylation and acetylation (Cereseto et al., 2005; Francis, Di Primio, Allouch, & Cereseto, 2011; Manganaro et al., 2010; Terreni et al., 2010). However, their relationship with IN dissociation is unknown. PARP-1 (poly-ADP ribose polymerase) has also been implicated in HIV

replication (Ariumi, Turelli, Masutani, & Trono, 2005; Gaken et al., 1996; Ha et al., 2001). PARP-1 is involved in the recognition and repair of single-stranded DNA regions by two mechanisms. PARP catalytic activation forms large poly (ADP ribose) polymer networks attached to chromatin (histones, topoisomerase I, and itself), which tends to dissociate PARylated proteins from DNA, regulate protein–protein interactions and repair enzyme activities. In addition, PARP binds directly to DNA repair complexes and controls base excision repair (BER; including XRCC1 and ligase III). PARP-1 has also been implicated in the nuclear proteasome activation responsible for histone degradation (Ullrich et al., 1999). Thus, a potential role of PARP-1 in HIV replication could be related to the dissociation process involving either PARylation or degradation of IN or key components of the integration complex. Because PARP inhibitors have recently emerged in the anticancer armamentarium (Rouleau, Patel, Hendzel, Kaufmann, & Poirier, 2010) and role of PARP in HIV replication remains controversial (Ariumi et al., 2005; Gaken et al., 1996; Ha et al., 2001; Kameoka et al., 2004; Kameoka et al., 2005; Siva & Bushman, 2002), further studies are warranted to determine the potential usefulness of PARP inhibitors as anti-HIV drugs.

Recent studies using siRNA screens have identified key DNA repair factors for integration (Brass et al., 2008; Espeseth et al., 2011; Konig et al., 2008; Zhou et al., 2008). The two most critical pathways for integration are BER and HR (Espeseth et al., 2011). The 14 BER genes identified (and detailed below) encode a coherent group of factors that can repair the proviral DNA gapped ends: (1) PARP1 might recognize the single-stranded gaps and recruit the BER complex; (2) the five glycosylases (MUTYH, NEIL2, NEIL3, NTHL1, and OGG1) can remove the two mismatched bases and generate abasic sites (see Fig. 3.3); (3) the major abasic site endonuclease (APEX1) can convert the abasic sites into breaks that would trim the 5′-ends of the vDNA; (4) any of the four identified repair polymerases (POLB, POLL, POLE, and POLI) can fill the gaps and remove the 5′-dRP residues resulting from the action of glycosylases with AP lyase activity; and (5) XRCC1 together with ligase III (LIG3) can finish the gap filling process by relegating the new DNA patch to the end of the vDNA. The importance of BER for proviral gap joining is consistent with earlier biochemical studies (Yoder & Bushman, 2000). It is notable that the glycosylases identified by genetic screening (Espeseth et al., 2011) are oxidative damage glycosylases, suggesting that oxidation of the bases at the ends of the vDNA might be important to initiate gap repair and allow full integration.

The implication of genes in the HR pathway (RAD51C, RAD51L3, RAD52, RAD50, MUS81, DMC1, RECQL4, and SMC6L1; Espeseth et al., 2011) suggests alternative pathways for the proviral DNA end processing. In which case, it remains to be shown whether the single-stranded gaps might be converted to double-strand breaks after breakage of the intact strand opposite to the gap. This possibility might explain the phosphorylation of histone gamma-H2AX in response to retroviral integration (Daniel et al., 2004) and the activation of DNA-dependent protein kinase during retroviral integration (Daniel, Katz, & Skalka, 1999). Insertion of the viral ends without the canonical 5-base-pair repeats might also reflect integration following double-strand breakage of the viral–host DNA junctions.

Activation of cellular processes involved in DNA damage response may also interfere with the normal vDNA fate. Components of NHEJ (non-homologous end-joining) including Ku70/Ku80, Ligase IV, and XRCC4 promote the production of 2-LTR circles (Jeanson et al., 2002; Li et al., 2001). Components of the MRN complex, including Mre11, NBS1, and Rad50 can induce the formation of 1-LTR circles through recombination (Kilzer et al., 2003).

6. CONCLUSION

During the past 30 years, AIDS evolved from a death sentence after only few months' survival to an asymptomatic disease with long-term remissions controlled by continuous drug treatments. The development of HAART played a crucial role in this transition and the approval of two INSTIs is a major step forward. The development of new, more efficient INIs that overcome drug resistance by viral mutations is warranted not only to cure and control the disease but also to prevent infections.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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