Resistance to novel drug classes

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Purpose of review

Understanding the mechanisms that underlie resistance development to novel drugs is essential to a better clinical management of resistant viruses and to prevent further resistance development and spread.

Recent findings

Integrase inhibitors and CCR5 antagonists are the more recent antiretroviral classes developed. The HIV-1 integrase, responsible for the chromosomal integration of the newly synthesized double-stranded viral DNA into the host genomic DNA, represents a new and important target; and two integrase inhibitors (INIs), raltegravir and elvitegravir, have been shown promising results in clinical trials. Viral entry is also an attractive step for the development of new drugs against HIV variants resistant to current antiretroviral drugs, and two CCR5 antagonists have been designed to inhibit HIV-1 binding to R5 co-receptor and are under clinical investigation.

Summary

Drug resistance to INIs occurs through the selection of mutations within HIV integrase. The kinetic of selection seems rapid and one mutation alone is able to confer resistance to integrase inhibitor, suggesting that this class of drug has a low genetic barrier. Two ways could explain the failure of the CCR5 antagonist class: a rapid outgrowth of pre-existing archived X4 virus or the selection of a resistance to CCR5 antagonists through amino acid changes in V3 loop.

Keywords

antiretrovirals, CCR5, integrase, new classes

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Introduction

Important progress has been made in the last 10 years in the development and the clinical use of drugs for treating HIV-1 infection. To date, nearly 25 antiretroviral drugs belonging to six drug classes have been licensed for the treatment of HIV-1. Most of them target the viral enzymes reverse transcriptase and protease, others the gp41, CCR5/gp120, and very recently the integrase. The combined use of all these drugs and the increased clinical experience have substantially improved the clinical management of HIV-1 infection in terms of delaying disease progression, prolonging survival, and improving quality of life [1]. Nevertheless, antiretroviral therapy can still fail to be fully suppressive and new viral variants emerge, thus allowing HIV-1 to become resistant to one or more drugs by accumulating mutations either alone or in multiple and complex patterns [2–12].

Integrase inhibitors and CCR5 antagonists represent the two more recent classes developed to block HIV replication. Understanding the mechanisms underlying resistance development to both existing and novel drugs is thus essential for a better clinical management of resistant viruses and for preventing further resistance development and spread.

Resistance to integrase inhibitors

HIV-1 integrase represents a new and important target of potential clinical relevance [13–15]; and two INIs, raltegravir and elvitegravir, have shown promising results in clinical trials. The first of these two inhibitors has been recently made available for clinical practice [16,17••, 18–20].

The HIV-1 integrase enzyme is responsible for the chromosomal integration of the newly synthesized double-stranded viral DNA into the host genomic DNA [21,22], enabling HIV-1 to establish a permanent genetic reservoir that can both initiate new virus production and replicate through cellular mitosis. HIV-1 integrase is a 32-kDa protein of 288 amino acids comprising three functional domains: the N-terminal domain (NTD; amino acids 1–49), the catalytic core domain (CCD; amino acids 50–212), and the C-terminal domain (CTD; amino

acids 213–288) [23]. The NTD contains a highly conserved zinc-binding $H_{12}H_{16}C_{40}C_{43}$ motif [22,24] involved in the stabilization folding and proper multimerization of the integrase subunits [25–27]. The CCD, which plays a critical role in integrase enzymatic activity, contains the catalytic $D_{64}D_{116}E_{152}$ motif, which is conserved in all retroviral integrases [22,24,28–32]. Several important residues in integrase are in contact with the human lens epithelium-derived growth factor (LEDGF/p75), which is an essential cellular cofactor for HIV integration, linking the integrase to chromatin [33–37].

The CTD has strong but nonspecific DNA-binding activity and is involved in the binding with viral and cellular DNA [38–41]. This domain, required for the integration reaction, is involved also in protein oligomerization and interactions with the reverse transcriptase [39].

Following reverse transcription, a multimeric form of integrase enzyme catalyzes two reactions: the first is a cleavage of two conserved nucleotides from the 3′ ends of both LTR strands of the viral cDNA (3′ processing) [42]. This reaction takes place within a nucleoprotein complex, referred to as the pre-integration complex (PIC), in the cytoplasm [43]. The PIC is transported through the nuclear pore to the nucleus where the second step (strand transfer) occurs. This consists of the insertion and the covalent ligation of the viral cDNA into the host genome [42,44,45].

As there is no human homologue of this enzyme, the HIV integrase represents a rational and important target for treating HIV infection and preventing AIDS. All integration steps can potentially be inhibited and each step can be considered a possible drug target [13–15].

To date, the strand transfer inhibitors (STIs) have been the most successful class of INIs, with the development of two clinically relevant inhibitors (elvitegravir and raltegravir) [16,17••,18–20].

As it is the case with other antiviral drugs, drug resistance to INIs occurs both *in vitro* and *in vivo* through the selection of mutations within HIV genome. So far, 64 integrase mutations (S17T, M50T, H51Y, T66AIK, L68IV, L74AIM, I72V, E92QG, Q95K, T97A, L101I, K111T, T112I, H114Y, S119GR, F121Y, T125K, A128T, E138AK, G140ACS, Y143CHR, Q146KP, S147G, Q148HKR, V151I, S153AY, M154I, N155HS, K156N, E157Q, K160DN, G163KR, V165I, R166S, E170A, S195C, V201I, I203M, T206S, S230NR, D232N, V249I, R263K, and C280Y) have already been associated with the resistance to all different INIs tested in in-vitro and/or in-vivo studies [6,12,17••,18,46,47,48•,49–54].

Most INI resistance mutations are in the vicinity of the putative INI binding pocket. Some resulted mutations were associated with a specific class of INIs, other with various inhibitors within the same STI class, and other with specific inhibitors within the same STI class, with a largely different magnitude of resistance [18,50, 53,54]. Forty integrase substitutions have been associated with the development of resistance to raltegravir and/or elvitegravir; some of them were also found *in vivo* in patients failing such INIs [6,17••,18,49,52,55–61]. For instance, N155H and Q148R/H/K have been identified as 'signature' resistance mutations in patients failing both raltegravir and elvitegravir, whereas Y143R/C was mainly associated with raltegravir, and E92Q and S147G were mainly associated with elvitegravir. Other resistance integrase mutations were observed in patients failing raltegravir and/or elvitegravir (L74M, T97A, E138K, G140SAC, and G163R). However, they had little or no effect on drug susceptibility *in vitro* in the absence of a primary 'signature' mutation, thus suggesting rather a secondary role for viral fitness rescue and/or increasing resistance [6,17••,18,49,52–61].

The relevance of all integrase mutations in clinical practice is yet to be defined in light of the lack of long-term follow-up of treated patients, the limited data about the prevalence of INI-associated mutations in INI-naïve patients [either untreated or treated with antiretrovirals (ARVs) not containing INI], and the scattered information about conservation and variability of HIV integrase in clean datasets. One report of two patients who had previously experienced virological failure on elvitegravir and were then switched to raltegravir suggested that these two drugs have clinically significant cross-resistance, as the antiviral response after the switch was not significant [62]. Some studies have recently started to analyze, within the public Los Alamos database, the prevalence of natural polymorphisms and mutations associated with INI resistance in the HIV-1 integrase, either in clade B [50] or from different subtypes of group M, N, and O viruses [63–65]. In addition, a single study added some information regarding the integrase variability in drug-naïve patients vs. ARV-treated patients with non-INI drugs (i.e., reverse transcriptase inhibitors and protease inhibitors) [66].

In addition to its obvious clinical relevance, the identification and characterization of conserved regions/residues within the HIV-1 integrase is of fundamental importance, which can help in designing new therapeutic strategies aimed at driving the virus to mutate at key amino acids that are crucial for the maintenance of sufficient viral fitness [50,63–66•].

Of the 64 mutations currently associated by in-vitro or in-vivo studies with resistance to the various INIs

currently discovered ([6,12,17••,18,46,47,48•,49–54], Stanford HIV Drug Resistance Database, http://hivdb.stanford.edu), 36 are completely absent in INI-naïve patients, either infected with HIV-1 B subtype (ART-naive or ART-treated [50,66], or non-B subtypes/group N and O [63–65]). This situation is true for all primary signature mutations (Y143HCR, S147G, Q148HKR, and N155H) or secondary mutations (H51Y, T66AK, L74A, E92Q, E138K, G140SAC, K160N, R166S, E170A, S230R, and R263K) found in patients failing raltegravir-containing or elvitegravir-containing regimens. Other resistance mutations (Q95K, F121Y, Q146P, and S153Y) known to reduce HIV-1 susceptibility *in vitro* to elvitegravir are also completely absent. Differently, some secondary mutations recently found in patients failing raltegravir-containing and/or elvitegravir-containing regimens [17••, 18] such as T66I, L68IV, E138A, E157Q, G163KR, and D232N mutations are rare (frequency <1%), whereas L74IM, T97A, S119GR, V151I, and I203M are present as natural polymorphisms with frequency of 1.3–6%; T206S and S230N are remarkably frequent (>10%).

The primary mutation T112I associated with resistance *in vitro* to the MK-2048, a potent second-generation INI able to inhibit some HIV-1-resistant variants generated with first-generation compounds [53], occurs at a low frequency. Six additional mutations associated with in-vitro resistance to INIs different than raltegravir or elvitegravir showed more than 10% variability (I72V, T125AV, M154I, V165I, and V201I).

All these data consistently show that all primary mutations associated with resistance to INIs clinically relevant today are absent or highly infrequent in INI-naïve patients.

However, for some secondary INI resistance-associated mutations, differences in prevalence between the distinct studies were observed. For instance, four integrase mutations (I84V, M154IL, and V165I, which are not associated with resistance to raltegravir or elvitegravir) showed a significant increase in the prevalence in HIV-1 B ART-treated patients compared with ART-naïve ones [66]. Two of them, previously associated with in-vitro resistance to other INIs (strand-transfer inhibitors as well as DNA-binding inhibitors and 3′ processing inhibitors) [50], M154I and V165I, occurred at 6% frequency in untreated patients, reaching 21.3 and 13.4%, respectively, in ART-treated patients. M154L was absent in ART-naïve patients and reached 5.7% in ART-treated ones. Similarly, I84V mutation occurred at 1.5% frequency in untreated patients, reaching at 5.7% frequency in ART-treated patients [66]. All these mutations within the Los Alamos Database, which mostly came from ART-naïve patients, had a frequency similar to that observed in HIV-1B subtype ART-naïve patients [50,65].

The mechanisms of this observed difference on the prevalence of some integrase mutations between drug-naïve and ART-treated patient populations need further investigations. It is conceivable that specific drug-pressure induced by protease inhibitors or in particular reverse transcriptase inhibitors (RTIs) may select or induce mutations also in different target regions within the same gene. For instance, very recent observations by us and by other groups indicate that there are some associations between integrase and reverse transcriptase resistance mutations in ART-failing patients [66–68], supporting the hypothesis of a close physical interaction between the viral integrase and reverse transcriptase and a potential co-evolution of some of their mutations. Further studies are required to elucidate this point of potentially relevant implications in clinical practice.

So far, in patients failing raltegravir-containing regimens, two main different pathways of raltegravir resistance have been generally associated with virological failure, each involving one signature primary mutation at positions N155 or Q148, plus some secondary mutations (L74M, E92Q, E138K, G140SAC, and G163R) important for viral fitness rescue and/or increasing resistance [17••,49,52, 53,55]. However, recent analyses suggest that, in addition to these common resistance profiles, there are other pathways associated with raltegravir resistance *in vivo*, involving E92Q, Y143HCR, or E157Q mutations [17••, 52,56–61].

The existence of distinct integrase resistance profiles is similar to what has been described for other ARV classes, such as nonnucleoside reverse transcriptase inhibitors (NNRTIs), nucleoside reverse transcriptase inhibitors (NRTIs), or protease inhibitors. However, it is unknown what are the determinants of the evolution toward these different profiles. The potential role of naturally occurring polymorphisms in HIV-1 integrase may have clinical and virological implications for INIs and is yet to be established in clinical practice.

It is possible that pre-existing integrase mutations, both occurring as natural polymorphisms and/or acquired/selected by previous virological failures with antiviral regimens different from INIs, may influence the integrase genetic pathways to develop resistance and could reduce the 'genetic barrier' and thus accelerate treatment failure to INIs.

In this context, HIV-1 group and subtype differences may also have an impact on evolution of resistance to INIs, as it has been described for protease inhibitors, NRTI, and NNRTIs [69–80]. Hackett *et al.*, by analyzing 1304 sequences from group M, N, and O viruses, have recently reported that some of the mutations associated with resistance to raltegravir and/or elvitegravir, such as

L74M, L74I, T97A, and E157Q, as well as others INI-resistance mutations (V165I, V201I, and T206S) occurred as natural polymorphisms (≥1%) and differently according to different HIV-1 subtype/CRF/group [62]. The significance of these polymorphic residues to the current generation of INIs is not yet known.

Interestingly, in this context, recent studies also showed promising results of efficacy of INIs in HIV-2. Despite a 40% heterogeneity between the HIV-1 and HIV-2 integrase genes, phenotypic susceptibility to raltegravir and elvitegravir in HIV-2 is similar to that of HIV-1 [81•,82], and virological and immunological response to a HAART regimen containing raltegravir in HIV-2-infected patients experiencing immunovirological failure to several previous ART lines has been reported [83]. Very recently, it has also been reported that HIV-1 and HIV-2 share similar INI resistance pathways. Indeed, both N155H and Q148KR mutations were observed in HIV-2-infected patients failing a raltegravir-containing regimen [84,85]. It should be noted that HIV-2 is naturally resistant to current NNRTIs and fusion inhibitors [86]; therefore, the so far, short-term immunological and virological efficacy of an INI-containing regimen also in heavily pretreated HIV-2-infected patients is really promising and clinically relevant.

Promising resistance data have recently been presented on S/GSK1349572, an investigational integrase inhibitor currently in phase II development [87]. Investigators evaluated the phenotypic activity of this agent *in vitro* against both viruses with site-directed integrase mutations and from clinical isolates, the latter drawn from patients virologically failing raltegravir-containing regimens. Encouragingly, although high-level resistance to raltegravir was common among the 30 clinical isolates with integrase mutations, four displayed a more than five-fold change in susceptibility to S/GSK1349572. These data suggest that S/GSK1349572 may have a role in treating patients who have experienced treatment failure with raltegravir; a study is ongoing to test this hypothesis.

Resistance to CCR5 antagonists

Less is known about resistance to CCCR5 antagonists. Viral entry is an attractive step for the development of new drugs against HIV variants resistant to current antiretroviral drugs and hopefully compounds in this family would also exhibit improved safety profiles relative to currently available antivirals. HIV gains entry into CD4-expressing cells through a series of sequential interactions between the envelope glycoprotein gp120 and the CD4 receptor and one of the two co-receptor molecules, CCR5 or CXCR4, which are expressed on the surface of target cells.

The chemokine receptors CCR5 and CXCR4 are the principal co-receptors for entry of HIV-1 into target cells [88,89]. Co-receptor selectivity is determined by genetic sequences within gp120, particularly on a highly variable and structurally flexible region termed 'V3' involved in co-receptor binding [90–92]. Two substances, maraviroc and vicriviroc, specifically designed to inhibit HIV-1 binding to R5 co-receptor are under clinical investigation in antiretroviral-naive or antiretroviral-treated patients. These two drugs exclusively inhibit the replication of R5-tropic HIV variants through an allosteric mechanism after binding to the transmembrane CCR5 co-receptor cavity. There are two ways to escape to CCR5 antagonists: selection of R5X4-tropic or X4-tropic viruses or development of resistance to such compounds.

Maraviroc-resistant viruses generated *in vitro* appear to be able to use either maraviroc-bound CCR5 or free CCR5 as a co-receptor for cell entry. This mechanism of resistance is characterized phenotypically by dose–response curves with a reduced maximal percentage inhibition (MPI).

In vivo, different sets of mutations in the V3 loop appear to play a role in resistance to maraviroc in R5 virus (G11S + I26V, S18G + A22T, A19S + I26V, I20F + A25D + I26V, I20F + Y21I) [93]. *In vitro*, the emergence of maraviroc resistance in HIV strain was associated with A21T and I28V mutations in the V3 region of gp120 [94••]. *In vivo*, emergence of vicriviroc resistance in an HIV-1 subtype C-infected patient was described, and experiences with chimeric envelopes demonstrated that changes in V3 loop were sufficient to confer vicriviroc resistance [95]. The V3 loop mutations at positions K10R, T12I, F21I, T23R, and G24E confer partial resistance to VCV, with the addition of S11P leading to complete resistance [95].

Regarding the facts that different set of mutations were described in patients failing a CCR5 antagonist regimen and the extreme genetic variability of the HIV envelope, it is possible to imagine that these mutations, which could be naturally present before introducing the CCR5 antagonists, may lead the virus to become resistant rapidly. In a recent study, it was possible to show that these resistance patterns to maraviroc are present in 7% of maraviroc-naive viruses [96].

In MOTIVATE trials, amino acid changes within the V3 loop sequence were observed for all resistant R5 viruses, with plateaus in maximal percentage inhibition less than 95%. Site-directed mutagenesis indicated the importance of the mutations in the V3 loop in the maraviroc resistance. The changes in amino acids I20F + A25D + I26V were both necessary and sufficient to confer resistance [97], and this set could be present in viruses in patients naive to CCR5 antagonists.

Similar to Lewis *et al.* [93], it was described that the V3 changes were concentrated in the stem and tip of the V3 loop and the base of the V3 loop appears to be largely conserved. The two invariant cysteines that form a disulfide bond to create a loop were found in this set of viruses. Mutations concentrated in the stem and tip of the V3 loop appear to play a key role in conferring the maraviroc-resistant phenotype in R5 virus. Changes in the V3 loop may enable the resistant virus to interact with the maraviroc-bound 'disrupted' form of the second extracellular loop (ECL2) of the CCR5 receptor.

There are different mechanisms for virological failure in patients receiving CCR5 antagonists. Although the MOTIVATE or the MERIT study was designed to include only patients with CCR5-tropic virus, patients included in the viral tropism studies changed from CCR5 tropic at screening to dual/mixed at baseline. A rapid outgrowth of pre-existing archived X4 virus is also demonstrated. Furthermore, a resistance to maraviroc in patients failing with R5 virus has been demonstrated. In the future, the lack of maraviroc efficacy on R5 strains could be eventually explained by the presence of amino acid implicated in resistance at baseline. Thus, the V3 loop genotyping could be proposed before introducing CCR5 antagonists.

Further studies are needed to conclude about the magnitude of the genetic barrier of CCR5 antagonists, the kinetic of resistance selection, and the magnitude of cross-resistance between compounds of this class.

Conclusion

The emergence and transmission of HIV-1 isolates resistant to existing antiretroviral drugs has serious clinical consequences. The development of resistance is, therefore, driving research to identify new drugs targeting novel steps in the HIV-1 replication cycle. Recent progress has been made in developing drugs targeting HIV-1 entry and integration. The addition of new drugs to the existing therapeutic arsenal will improve treatment options and clinical prospects particularly for those patients failing current drug regimens based primarily on combinations of RTI and protease inhibitors. Despite the negative impact of drug resistance in the clinic, understanding resistance mechanisms provides a powerful tool to aid the discovery and development of new HIV-1 therapies.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 549).

1 Antiretroviral Therapy Cohort Collaboration. Life expectancy of individuals on combination antiretroviral therapy in high-income countries: a collaborative analysis of 14 cohort studies. *Lancet* 2008; 372:293–299.

2 Ceccherini-Silberstein F, Svicher V, Sing T, *et al.* Characterization and structural analysis of novel mutations in human immunodeficiency virus type 1 reverse transcriptase involved in the regulation of resistance to nonnucleoside inhibitors. J Virol 2007; 81:11507–11519.

3 Clavel F, Hance AJ. HIV drug resistance. N Engl J Med 2004; 350:1023–1035.

4 Cozzi-Lepri A, Ruiz L, Loveday L, *et al.* Thymidine analogue mutation profiles: factors associated with acquiring specific profiles and their impact on the virological response to therapy. Antivir Ther 2005; 10:791–802.

5 Hanna G, Johnson V, Kuritzkes D, *et al.* Patterns of resistance mutations selected by treatment of human immunodeficiency virus type 1 infection with zidovudine, didanosine, and nevirapine. J Infect Dis 2000; 181:904–911.

6 Johnson VA, Brun-Vézinet F, Clotet B, *et al.* Update of the drug resistance mutations in HIV-1: spring 2008. Top HIV Med 2008; 16:62–68.

7 Larder B, Kemp S. Multiple mutations in HIV-1 reverse transcriptase confer high-level resistance to zidovudine (AZT). Science 1989; 246:1155–1158.

8 Perno CF, Cozzi-Lepri A, Balotta C, *et al.* Secondary mutations in the protease region of human immunodeficiency virus and virologic failure in drug-naïve patients treated with protease inhibitor-based therapy. J Infect Dis 2001; 184:983–991.

9 Rhee SY, Taylor J, Wadhera G, *et al.* Genotypic predictors of human immunodeficiency virus type 1 drug resistance. Proc Natl Acad Sci U S A 2006; 103:17355–17360.

10 Svicher V, Ceccherini-Silberstein F, Erba F, *et al.* Novel human immunodeficiency virus type 1 protease mutations potentially involved in resistance to protease inhibitors. Antimicrob Agents Chemother 2005; 49:2015–2025.

11 Svicher V, Sing T, Santoro MM, *et al.* Involvement of novel human immunodeficiency virus type 1 reverse transcriptase mutations in the regulation of resistance to nucleoside inhibitors. J Virol 2006; 80:7186–7198.

12 Shafer RW, Schapiro JM. HIV-1 drug resistance mutations: an updated framework for the second decade of HAART. AIDS Rev 2008; 10:67–84.

13 Lataillade M, Kozal MJ. The hunt for HIV-1 integrase inhibitors. AIDS Patient Care STDS 2006; 20:489–501.

14 Pommier Y, Johnson AA, Marchand C. Integrase inhibitors to treat HIV/AIDS. Nat Rev Drug Discov 2005; 4:236–248.

15 Semenova EA, Marchand C, Pommier Y. HIV-1 integrase inhibitors: update and perspectives. Adv Pharmacol 2008; 56:199–228.

16 Grinsztejn B, Nguyen BY, Katlama C, *et al.* Safety and efficacy of the HIV-1 integrase inhibitor raltegravir (MK-0518) in treatment experienced patients with multidrug resistant virus: a phase II randomised controlled trial. Lancet 2007; 369:1261–1269.

17 •• Cooper DA, Steigbigel RT, Gatell JM, *et al.* Subgroup and resistance analyses of raltegravir for resistant HIV-1 infection. *N Engl J Med* 2008; 359:355–365.
This study shows that, at 48 weeks, 105 of 462 raltegravir recipients (23%) had virologic failure. Genotyping was performed in 94 raltegravir recipients with virologic failure. Integrase mutations known to be associated with phenotypic resistance to raltegravir arose during treatment in 64 patients (68%).

18 McColl DJ, Fransen S, Gupta S, *et al.* Resistance and cross-resistance to first generation integrase inhibitors: insights from a phase 2 study of elvitegravir (GS-9137). Antivir Ther 2007; 12:S11.

19 Steigbigel RT, Cooper DA, Kumar PN, *et al.* Raltegravir with optimized background therapy for resistant HIV-1 infection. N Engl J Med 2008; 359:339–354.

20 Zolopa AR, Mullen M, Berger D, Ruane P, *et al.* The HIV integrase inhibitor GS-9137 demonstrates potent antiretroviral activity in treatment-experienced patients. *14th Conference on Retrovirus and Opportunistic Infection*; 25–28 February 2007; Los Angeles, CA, USA; abstract 143LB.

21 Coffin JM, Hughes SH, Varmus HE. Retroviruses. New York: Cold Spring Harbor Laboratory Press; 1997.

22 Rice P, Craigie R, Davies DR. Retroviral integrases and their cousins. Curr Opin Struct Biol 1996; 6:76–83.

23 Engelman A, Craigie R. Identification of conserved amino acid residues critical for human immunodeficiency virus type 1 integrase function *in vitro*. J Virol 1992; 66:6361–6369.

24 Polard P, Chandler M. Bacterial transposases and retroviral integrases. Mol Microbiol 1995; 15:13–23.

25 Burke CJ, Sanyal G, Bruner MW, *et al.* Structural implications of spectroscopic characterization of a putative zinc finger peptide from HIV-1 integrase. J Biol Chem 1992; 267:9639–9644.

26 Lee SP, Xiao J, Knutson JR, *et al.* Zn^{2+} promotes the self-association of human immunodeficiency virus type-1 integrase *in vitro*. Biochemistry 1997; 36:173–180.

27 Zheng R, Jenkins TM, Craigie R. Zinc folds the N-terminal domain of HIV-1 integrase, promotes multimerization, and enhances catalytic activity. Proc Natl Acad Sci U S A 1996; 93:13659–13664.

28 Avidan O, Hizi A. Expression and characterization of the integrase of bovine immunodeficiency virus. Virology 2008; 371:309–321.

29 Kulkosky J, Katz RA, Merkel G, Skalka AM. Activities and substrate specificity of the evolutionarily conserved central domain of retroviral integrase. Virology 1995; 206:448–456.

30 Bouyac-Bertoia M, Dvorin JD, Fouchier RA, *et al.* HIV-1 infection requires a functional integrase NLS. Mol Cell 2001; 7:1025–1035.

31 Berthoux L, Sebastian S, Muesing MA, Luban J. The role of lysine 186 in HIV-1 integrase multimerization. Virology 2007; 364:227–236.

32 Wang JY, Ling H, Yang W, Craigie R. Structure of a two-domain fragment of HIV-1 integrase: implications for domain organization in the intact protein. EMBO J 2001; 20:7333–7343.

33 Busschots K, Voet A, De Maeyer M, *et al.* Identification of the LEDGF/p75 binding site in HIV-1 integrase. J Mol Biol 2007; 365:1480–1492.

34 Cherepanov P, Sun ZY, Rahman S, *et al.* Solution structure of the HIV-1 integrase-binding domain in LEDGF/p75. Nat Struct Mol Biol 2005; 12:526–532.

35 Hombrouck A, De Rijck J, Hendrix J, *et al.* Virus evolution reveals an exclusive role for LEDGF/p75 in chromosomal tethering of HIV. PLoS Pathog 2007; 3:e47.

36 Maertens G, Cherepanov P, Pluymers W, *et al.* LEDGF/p75 is essential for nuclear and chromosomal targeting of HIV-1 integrase in human cells. J Biol Chem 2003; 278:33528–33539.

37 Rahman S, Lu R, Vandegraaff N, *et al.* Structure-based mutagenesis of the integrase-LEDGF/p75 interface uncouples a strict correlation between *in vitro* protein binding and HIV-1 fitness. Virology 2007; 357:79–90.

38 Engelman A, Hickman AB, Craigie R. The core and carboxyl-terminal domains of the integrase protein of human immunodeficiency virus type 1 each contribute to nonspecific DNA binding. J Virol 1994; 68:5911–5917.

39 Lutzke RA, Plasterk RH. Structure-based mutational analysis of the C-terminal DNA-binding domain of human immunodeficiency virus type 1 integrase: critical residues for protein oligomerization and DNA binding. J Virol 1998; 72:4841–4848.

40 Lutzke RA, Vink C, Plasterk RH. Characterization of the minimal DNA-binding domain of the HIV integrase protein. Nucleic Acids Res 1994; 22:4125–4131.

41 Vink C, Oude Groeneger AM, Plasterk RH. Identification of the catalytic and DNA-binding region of the human immunodeficiency virus type I integrase protein. Nucleic Acids Res 1993; 21:1419–1425.

42 Engelman A, Mizuuchi K, Craigie R. HIV-1 DNA integration: mechanism of viral DNA cleavage and DNA strand transfer. Cell 1991; 67:1211–1221.

43 Miller MD, Farnet CM, Bushman FD. Human immunodeficiency virus type 1 preintegration complexes: studies of organization and composition. J Virol 1997; 71:5382–5390.

44 Mulder LC, Chakrabarti LA, Muesing MA. Interaction of HIV-1 integrase with DNA repair protein hRad18. J Biol Chem 2002; 277:27489–27493.

45 Yoder K, Bushman FD. Repair of gaps in retroviral DNA integration intermediates. J Virol 2000; 74:11191–11200.

46 Fikkert V, Hombrouck A, Van Remoortel B, *et al.* Multiple mutations in HIV-1 integrase confer resistance to the clinical trial drug S-1360. AIDS 2004; 18:2019–2028.

47 Hazuda D, Anthony N, Gomez R, *et al.* A naphthyridine carboxamide provides evidence for discordant resistance between mechanistically identical inhibitors of HIV-1 integrase. Proc Natl Acad Sci U S A 2004; 101:11233–11238.

48 • Ceccherini-Silberstein F, Van Baelen K, Armenia D, *et al.* Novel HIV-1 integrase mutations, found as minority quasispecies in patients naïve to integrase inhibitors, are associated with decreased susceptibility to integrase inhibitors *in vitro*. *15th Conference on Retrovirus and Opportunistic Infection*; 3–6 February 2008; Boston, MA, USA; abstract 876.
This cross-sectional study shows that mutations associated with resistance to integrase inhibitors are present at low prevalence as minority species in 49 integrase-naive HIV-1 subtype B-infected patients.

49 Hazuda DJ, Miller MD, Nguyen BY, Zhao J, for the P005 Study Team. Resistance to the HIV-integrase inhibitor raltegravir: analysis of protocol 005, a Phase II study in patients with triple-class resistant HIV-1 infection. Antivir Ther 2007; 12:S10.

50 Lataillade M, Chiarella J, Kozal MJ. Natural polymorphism of the HIV-1 integrase gene and mutations associated with integrase inhibitor resistance. Antivir Ther 2007; 12:563–570.

51 Shimura K, Kodama E, Sakagami Y, *et al.* Broad antiretroviral activity and resistance profile of the novel human immunodeficiency virus integrase inhibitor elvitegravir (JTK-303/GS-9137). J Virol 2008; 82:764–774.

52 Steigbigel R, Kumar P, Eron J, *et al.* Results of BENCHMRK-2, a phase III study evaluating the efficacy and safety of MK-0518, a novel HIV-1 integrase inhibitor, in patients with triple-class resistant virus. *14th Conference on Retrovirus and Opportunistic Infection*; 25–28 February 2007; Los Angeles, CA, USA; abstract 105bLB.

53 Wai J, Fisher T, Embrey M, *et al.* Next generation of inhibitors of HIV-1 integrase strand transfer inhibitor: structural diversity and resistance profiles. *14th Conference on Retrovirus and Opportunistic Infection*; 25–28 February 2007; Los Angeles, CA, USA; abstract 87.

54 Goethals O, Clayton R, Van Ginderen M, *et al.* Resistance mutations in HIV-1 integrase selected with Elvitegravir confer reduced susceptibility to a wide range of integrase inhibitors. J Virol 2008; 82:10366–10374.

55 Fransen S, Gupta S, Danovich R, *et al.* Loss of raltegravir susceptibility in treated patients is conferred by multiple nonoverlapping genetic pathways [abstract 7]. Antivir Ther 2008; 13 (Suppl 3):A9.

56 Malet I, Delelis O, Valantin MA, *et al.* Mutations associated with failure of raltegravir treatment affect integrase sensitivity to the inhibitor *in vitro*. Antimicrob Agents Chemother 2008; 52:1351–1358.

57 Goodman D, Hluhanich R, Waters J, *et al.* Integrase inhibitor resistance involves complex interactions among primary and secondary resistance mutations: a novel mutation L68V/I associates with E92Q and increases resistance [abstract 13]. Antviral Ther 2008; 13 (Suppl 3):A15.

58 Hatano H, Lampiris H, Huang W, *et al.* Virological and immunological outcomes in a cohort of patients failing integrase inhibitors [abstract 10]. Antivir Ther 2008; 13 (Suppl 3):A12.

59 Katlama C, Caby F, Andrade R, *et al.* Virological evolution in HIV treatment-experienced patients with raltegravir-based salvage regimens [abstract 11]. Antivir Ther 2008; 13 (Suppl 3):A13.

60 Da Silva D, Pellgrin I, Anies G, *et al.* Mutational patterns in the HIV-1 integrase related to virological failures on raltegravir-containing regimens [abstract 12]. Antivir Ther 2008; 13 (Suppl 3):A14.

61 Ceccherini-Silberstein F, Armenia D, D'Arrigo R, *et al.* Virological response and resistance in multiexperienced patients treated with raltegravir [abstract 18]. Antivir Ther 2008; 13 (Suppl 3):A20.

62 DeJesus E, Cohen C, Elion R, *et al.* First report of raltegravir (RAL, MK-0518) use after virologic rebound on elvitegravir (EVT, GS 9137). *4th International AIDS Society Conference on HIV Pathogenesis, Treatment, and Prevention*; 22–25 July 2007; Sydney, Australia; abstract TUPEB032.

63 Hackett J Jr, Harris B, Holzmayer V, *et al.* Naturally occurring polymorphisms in HIV-1 group M, N, and O integrase: implications for integrase inhibitors. *15th Conference on Retrovirus and Opportunistic Infection*; 3–6 February 2008; Boston, MA, USA; abstract 872.

64 Myers RE, Pillay D. HIV Analysis of natural sequence variation and covariation in human immunodeficiency virus type 1 integrase. J Virol 2008; 82:9228–9235.

65 Rhee SY, Liu TF, Kiuchi M, *et al.* Natural variation of HIV-1 group M integrase: implications for a new class of antiretroviral inhibitors. Retrovirology 2008; 5:74.

66 Ceccherini-Silberstein F, Malet I, Fabeni L, *et al.* Specific mutations related to resistance to HIV-1 integrase inhibitors are associated with reverse transcriptase mutations in HAART-treated patients. Antivir Ther 2007; 12:S6.

67 Sander O, Altmann A, Lenguauer T. Computational analysis of covariation and interactions between HIV-1 reverse transcriptase and integrase. *6th European HIV Drug Resistance Workshop*; 2008; 10–14 June 2008; Sitges, Spain; abstract 108.

68 Van Eygen V, Van Marck H, Smits V, *et al.* Identification of residues in HIV-1 integrase, RNaseH and the RT connection domain associated with the presence of thymidine analogue-associated resistance. *6th European HIV Drug Resistance Workshop*; 10–14 June 2008, Sitges, Spain; abstract 53.

69 Brenner BG, Oliveira M, Doualla-Bell F, *et al.* HIV-1 subtype C viruses rapidly develop K65R resistance to tenofovir in cell culture. AIDS 2006; 20:F9–F13.

70 Brenner B, Turner D, Oliveira M, *et al.* A V106M mutation in HIV-1 clade C viruses exposed to efavirenz confers cross-resistance to nonnucleoside reverse transcriptase inhibitors. AIDS 2003; 17:F1–F5.

71 Calazans A, Brindeiro R, Brindeiro P, *et al.* Low accumulation of L90M in protease from subtype F HIV-1 with resistance to protease inhibitors is caused by the L89M polymorphism. J Infect Dis 2005; 191:1961–1970.

72 Doualla-Bell F, Avalos A, Brenner B, *et al.* High prevalence of the K65R mutation in human immunodeficiency virus type 1 subtype C isolates from infected patients in Botswana treated with didanosine-based regimens. Antimicrob Agents Chemother 2005; 50:4182–4185.

73 Grossman Z, Istomin V, Averbuch D, *et al.* Genetic variation at NNRTI resistance-associated positions in patients infected with HIV-1 subtype C. AIDS 2004; 18:909–915.

74 Grossman Z, Paxinos EE, Averbuch D, *et al.* Mutation D30N is not preferentially selected by human immunodeficiency virus type 1 subtype C in the development of resistance to nelfinavir. Antimicrob Agents Chemother 2004; 48:2159–2165.

75 Gupta RK, Chrystie IL, O'Shea S, *et al.* K65R and Y181C are less prevalent in HAART-experienced HIV-1 subtype A patients. AIDS 2005; 19:1916–1919.

76 Kantor R, Katzenstein DA, Efron B, *et al.* Impact of HIV-1 subtype and antiretroviral therapy on protease and reverse transcriptase genotype: results of a global collaboration. PLoS Med 2005; 2:e112.

77 Loemba H, Brenner B, Parniak MA, *et al.* Genetic divergence of human immunodeficiency virus type 1 Ethiopian clade C reverse transcriptase (RT) and rapid development of resistance against nonnucleoside inhibitors of RT. Antimicrob Agents Chemother 2002; 46:2087–2094.

78 Miller MD, Margot N, McColl D, Cheng AK. K65R development among subtype C HIV-1-infected patients in tenofovir DF clinical trials. AIDS 2007; 21:265–266.

79 Soares EA, Santos RP, Pellegrini JA, *et al.* Epidemiologic and molecular characterization of human immunodeficiency virus type 1 in southern Brazil. J Acquir Immune Defic Syndr 2003; 34:520–526.

80 Taylor BS, Sobieszczyk ME, McCutchan FE, Hammer SM. The challenge of HIV-1 subtype diversity. N Engl J Med 2008; 358:1590–1602.

81 • Roquebert B, Damond F, Collin G, *et al.* HIV-2 integrase gene polymorphism and phenotypic susceptibility of HIV-2 clinical isolates to the integrase inhibitors raltegravir and elvitegravir in vitro. J Antimicrob Chemother 2008; 62:914–920.
This cross-sectional study shows that despite 40% heterogeneity between the HIV-1 and HIV-2 integrase genes, the phenotypic susceptibility of clinical HIV-2 isolates to INIs was similar to that of HIV-1.

82 Van Baelen K, Van Eygen V, Rondelez E, Stuyver LJ. Clade-specific HIV-1 integrase polymorphisms do not reduce raltegravir and elvitegravir phenotypic susceptibility. AIDS 2008; 22:1877–1880.

83 Damond F, Lariven S, Roquebert B, *et al.* Virological and immunological response to HAART regimen containing integrase inhibitors in HIV-2-infected patients. AIDS 2008; 22:665–666.

84 Garrett N, Xu L, Smit E, *et al.* Raltegravir treatment response in an HIV-2 infected patient: a case report. AIDS 2008; 22:1091–1092.

85 Roquebert B, Blum L, Collin G, *et al.* Selection of the Q148R integrase inhibitor resistance mutation in a failing raltegravir containing regimen. AIDS 2008; 22:2045–2046.

86 Withrouw M, Pannecouque C, Switzer WM, *et al.* Susceptibility of HIV-2, SIV and SHIV to various anti-HIV-1 compounds: implications for treatment and postexposure prophylaxis. Antivir Ther 2004; 9:57–65.

87 Underwood M, Johns B, Sato A, *et al.* S/GSK1349572: a next generation integrase inhibitor with activity against integrase inhibitor-resistant clinical isolates from patients experiencing virologic failure while on raltegravir therapy. *IAS 2009*; 19–22 July 2009; Cape Town, South Africa; #WEPEA098.

88 Berger EA, Murphy PM, Farber JM. Chemokine receptors as HIV-1 coreceptors: roles in viral entry, tropism, and disease. Annu Rev Immunol 1999; 17:657–700.

89 Poveda E, Briz V, Quinones-Mateu M, Soriano V. HIV tropism: diagnostic tools and implications for disease progression and treatment with entry inhibitors. AIDS 2006; 20:1359–1367.

90 Briggs DR, Tuttle DL, Sleasman JW, Goodenow MM. Envelope V3 amino acid sequence predicts HIV-1 phenotype (co-receptor usage and tropism for macrophages). AIDS 2000; 14:2937–2939.

91 Jensen MA, Li FS, van 't Wout AB, *et al.* Improved coreceptor usage prediction and genotypic monitoring of R5-to-X4 transition by motif analysis of human immunodeficiency virus type 1 env V3 loop sequences. J Virol 2003; 77:13376–13388.

92 Fouchier RA, Groenink M, Kootstra NA, *et al.* Phenotype-associated sequence variation in the third variable domain of the human immunodeficiency virus type 1 gp120 molecule. J Virol 1992; 66:3183–3187.

93 Lewis M, Simpson P, Whitcomb J, *et al.* Changes in V3 loop sequence associated with failure of maraviroc treatment in patients enrolled in the MOTIVATE 1 and 2 Trials. *15th Conference on Retroviruses and Opportunistic Infections*; 3–6 February 2008; Boston, MA, USA.

94 •• Westby M, Smith-Burchnell C, Mori J, *et al.* Reduced maximal inhibition in phenotypic susceptibility assays indicates that viral strains resistant to the CCR5 antagonist maraviroc utilize inhibitor-bound receptor for entry. J Virol 2007; 81:2359–2371.
This study shows that maraviroc-resistant virus derived from isolates remained CCR5 tropic. Strain-specific mutations were identified in the V3 loop of maraviroc resistant virus. The plateaus are consistent with the virus having acquired the ability to utilize maraviroc-bound receptor for entry.

95 Tsibris AM, Sagar M, Gulick RM, *et al.* In vivo emergence of vicriviroc resistance in an HIV-1 subtype C-infected subject. J Virol 2008; 82:8210–8214.

96 Soulié C, Malet I, Lambert-Niclot S, *et al.* Primary genotypic resistance of HIV-1 to CCR5 antagonists in CCR5 antagonist treatment-naive patients. AIDS 2008; 22:2212–2214.

97 Mori J, Lewis M, Simpson P, *et al.* Characterization of maraviroc resistance in patients failing treatment with CCR5-tropic HIV-1 in MOTIVATE 1 and MOTIVATE 2. *XVI International HIV Drug Resistance Workshop*; 12–16 June 2007; Barbados, West Indies.