

Point Mutations Associated with HIV-1 Drug Resistance, Evasion of the Immune Response and AIDS Pathogenesis

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1. Introduction

Point mutations within the human immunodeficiency virus-type 1 (HIV-1) genome confer resistance to antiretroviral drugs. The mutations also help the virus to evade the immune system response and influence transmission and progression of the disease, collectively called acquired immune deficiency syndrome (AIDS) pathogenesis. This chapter presents an up-to-date critical review of the literature and provides a synthesis of the current understanding of HIV-1 point mutations in relation to drug resistance, evasion of the immune system and AIDS pathogenesis. First, to prime the reader, the chapter briefly describes organization and function of the HIV-1 genes. It then pinpoints salient point mutations within specific HIV-1 genes associated with resistance to current antiretroviral drugs. In particular, it pays attention to the *env* gene and its product, the surface envelope (Env) glycoprotein because the Env protein mediates viral entry, tropism and disease progression. Thus the role and frequencies of point mutations within the *env* gene is related to HIV-1 transmission dynamics and progression to AIDS. The *env* gene and Env protein are further unpacked to provide a better understanding of the role point mutations within this gene play in helping the virus to evade antibody mediated immune response. Finally, drawing mainly from our recent research work, the chapter proposes an aptamer-based strategy for the design of desirable approaches that can help circumvent HIV-1 point mutations and delay or diminish drug resistance.

2. Organization and function of the HIV-1 genes

The genome of HIV-1, unlike other retroviruses is made up of a single coding RNA strand of 9.2 kb in length and is far more complex than those of other retroviruses. The genome of most retroviruses is diploid. It is made up of two identical RNA strands with a plus (+) polarity. All retroviruses have the *env*, *gag* and *pol* genes, but HIV-1 has 6 additional regulatory and accessory genes (Figure 1), which produces 14 proteins (Table 1). The *env* gene encodes the envelope (Env) protein. The Env protein is made up of two polypeptides derived from a gp160 single precursor; the transmembrane (TM) gp41 and the outer surface

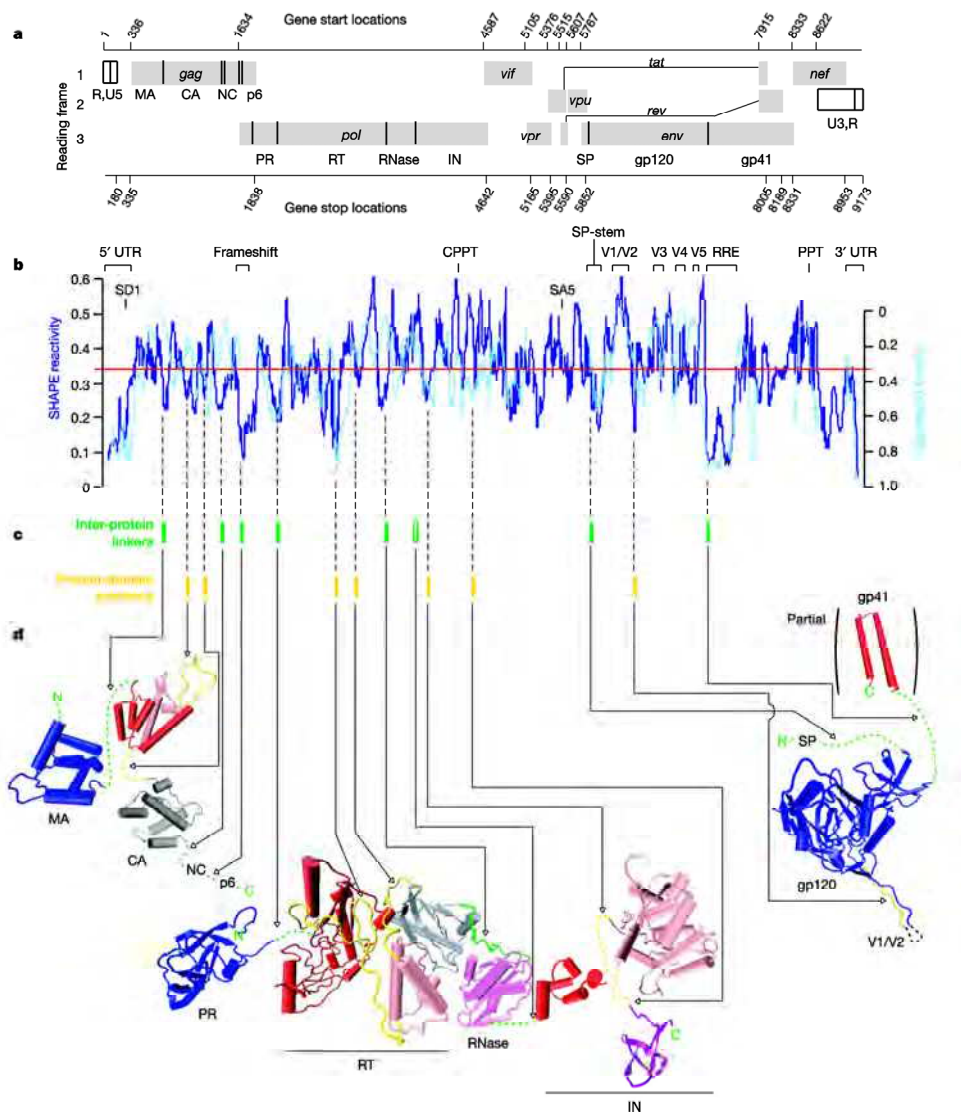


Fig. 1. Organization and function of the HIV-1 genome in relation to the proteins coded. (a) Protein coding region are shown as grey boxes. (b) Comparison of the pairing probability of nucleotides in the overall HIV-1 structure using 2'-hydroxyl acylation analysed by primer extension (SHAPE) reactivity. (c) Inter-protein linkers and unstructured peptide loops that link protein domains. (d) Three-dimensional structures of large and key HIV-1 proteins encoded by *env* and *pol* genes, respectively. Reproduced from (Watts et al., 2009) with permission from Nature Publishing Group (Copyright Clearance Centre License Number: 2754150202433)

(SU) gp120 (Figure 1). For a mature virion, on the inner leaflet of the envelope there are proteolytically processed *gag* gene products: a p17 matrix (MA) protein and a p24 capsid (CA) protein that form the core of the particle and an RNA-binding p7 nucleocapsid (NC) protein (Figure 1 and Table 1). Other proteins present in the core of the virion particle are derived from the *pol* gene and perform enzymatic functions. HIV-1 gp120 and gp41 (*env* gene products) and *pol* gene products, reverse transcriptase (RT) and integrase (IN) are involved in early stages of the HIV life cycle and are primary targets of therapeutic drugs. Env, RT and IN are targets of antiretroviral drugs called entry inhibitors (EI), reverse transcriptase inhibitors (RTI) and integrase inhibitors (INI), respectively. Another *pol* gene product called protease (PR) is involved in later stages of the HIV life cycle and it is also a target of a class of antiretroviral drugs called protease inhibitors. Other HIV-1 genes encode auxiliary proteins (Figure 1).

Gene	Protein encoded	Function
<i>gag</i>	Gag MA	Membrane anchoring; env interaction; nuclear transport of viral core
<i>gag</i>	Gag CA	Core capsid
<i>gag</i>	Gag NC	Binds RNA and Vpr, respectively
<i>pol</i>	Protease (PR)	<i>gag/pol</i> cleavage
<i>pol</i>	Reverse Transcriptase (RT)	Reverse transcription
<i>pol</i>	RNase H	RNase H activity
<i>pol</i>	Integrase (IN)	DNA provirus integration
<i>env</i>	Env (gp120 and gp41)	gp120 binds CD4 receptor and CXCR4/CCR5 co-receptors , gp41 mediates fusion
<i>tat</i>	Tat	Viral transcription activator
<i>rev</i>	Rev	RNA transport, stability and utilization factor (phosphoprotein)
<i>vif</i>	Vif	Promotes virion maturation and infectivity
<i>vpr</i>	Vpr	Promotes nuclear localization of preintegration complex, inhibits cell division, arrest infected cells at G2/M
<i>vpu</i>	Vpu	Promotes extracellular release of viral particles. Important for viral assembly and budding
<i>nef</i>	Nef	CD4 and class I downregulation

Table 1. HIV-1 genes, proteins coded by the respective genes and their functions

3. Point mutations and HIV-1 drug resistance

Antiretroviral drugs are currently the cornerstone of controlling HIV-1 in infected individuals. To date, 24 antiretroviral drugs have been approved and licensed for the treatment of HIV-1 infection. Thirteen of these drugs are reverse transcriptase inhibitors (nine nucleoside and four non-nucleoside RT inhibitors); nine are protease inhibitors; a fusion inhibitor; a CCR5 inhibitor and an integrase inhibitor. However, despite the use of

current antiretroviral drugs, HIV-1 can still persist in infected individuals by accumulating mutations, which make it resistant to one or more drugs. The antiretroviral drug resistant mutations can be identified by one or more of the following: (a) *in vitro* passage experiments or site directed mutagenesis studies (Clark *et al.*, 2006); (b) mutagenically separated PCR (Frater *et al.*, 2001); (c) drug susceptibility testing (Petropoulos *et al.*, 2000; Svicher *et al.*, 2011); (d) nucleotide sequencing of viruses from patients failing a specific drug (McNicholas *et al.*, 2011; Shulman *et al.*, 2004); (e) correlation studies between genotype at baseline and virologic response in patients exposed to a specific antiretroviral drug (Demeter *et al.*, 2008). Currently, more than 200 mutations in the HIV-1 *pol* and *env* genes associated with resistance to current antiretroviral drugs, which include reverse transcriptase inhibitors (RTIs), protease inhibitors (PIs), integrase inhibitors (INI), fusion inhibitors and attachment inhibitors respectively, have been identified (V. A. Johnson *et al.*, 2010; Shafer & Schapiro, 2008).

3.1 Point mutations associated with resistance to NRTIs

HIV-1 RT is a heterodimer made up of two subunits, p66 and p51 (di Marzo Veronese *et al.*, 1986) and its crystal structure has been solved (Arnold *et al.*, 1992; Kohlstaedt *et al.*, 1992b). RT is important in the HIV-1 life cycle because it orchestrates synthesis of a linear double-stranded DNA copy of a viral single-stranded RNA genome. The knowledge that HIV-1 is a retrovirus immediately pinpointed RT synthesis of proviral DNA as a target for a class of antiretroviral drugs called RTIs. Indeed, RTIs including the prototype azidothymidine (AZT) also known as zidovudine (ZDV), were the first to be approved for clinical use (Fischl *et al.*, 1987), and remains the mainstay of HIV-1 therapy. Nearly half of current antiretroviral drugs target the polymerase activity of RT. RTIs belong to one of the two broad categories: nucleoside RT inhibitors (NRTIs) and non-nucleoside RT inhibitors (NNRTIs).

NRTIs are structural analogues of natural substrate of DNA synthesis. They lack the 3'-hydroxyl group (3'-OH) and hence they block HIV-1 replication by acting as chain terminators when incorporated into a viral DNA by RT. Two basic NRTI resistance mechanisms are exclusion and excision. The exclusion mechanism involves enhanced discrimination at the time the NRTI tri-phosphate is incorporated in the nascent DNA strand. The M184V/I point mutation (Figure 2 and Table 2), which selectively reduces incorporation of abacavir (ABC); emtricitabine (FTC) and lamivudine (3TC) NRTIs into a nascent DNA chain by steric hindrance (Gao *et al.*, 2000; Sarafianos *et al.*, 1999) is a common example of the exclusion mechanism. M184V point mutation causes a median 1.5-fold and 3.0-fold reduction in susceptibility to didanosine (ddI) and ABC, respectively in the PhenoSenseGT™ assay (Petropoulos *et al.*, 2000; Rhee *et al.*, 2004). However several clinical trials have shown that ABC and ddI retain clinical activity in the presence of M184V point mutation (Brun-Vezinet *et al.*, 2003; J. J. Eron, Jr. *et al.*, 2007; Lanier *et al.*, 2004a; Marcelin *et al.*, 2005; Molina *et al.*, 2005; M. A. Winters *et al.*, 2003). The phenotypic and clinical significance of M184V is influenced by the presence or absence of other NRTI resistance mutations (Shafer & Schapiro, 2008). For instance, the presence of K65R or L74V (J. Eron, Jr. *et al.*, 2006; Gallant *et al.*, 2006; Moyle *et al.*, 2005) point mutations (Figure 2 and Table 2) in addition to the M184V is sufficient for high level resistance to ddI and ABC (Rhee *et al.*, 2004), particularly in patients with non-subtype B clades (Harrigan *et al.*, 2000; Lanier *et al.*, 2004a; Svarovskaia *et al.*, 2007; M. A. Winters *et al.*, 1997). K65R has emerged more rapidly during the *in vitro* passage of subtype C compared with subtype B isolates in the presence of

increasing concentrations of TDF (Brenner *et al.*, 2006). The emergence of K65R mutation is suppressed maximally in regimens containing AZT compared with d4T (Roge *et al.*, 2003). Thus, the K65R point mutation commonly causes intermediate resistance to tenofovir (TDF), ABC, ddI, 3TC and FTC (Table 2), low level resistance to d4T, and increased susceptibility to AZT (Antinori *et al.*, 2007; Lanier *et al.*, 2004b). There is a body of evidence suggesting that K65R mutation occurs more commonly in low-income countries when patients with non-subtype B HIV-1 strains are treated with d4T/ddI and d4T/3TC (Doualla-Bell *et al.*, 2006; Hawkins *et al.*, 2009) or TDF/3TC (Rey *et al.*, 2009). In comparison to K65R, L74V point mutation causes intermediate resistance to ddI and ABC, and a slight increase in susceptibility to AZT and TDF (Rhee *et al.*, 2006b). L74V and K65R mutations rarely occur in the same viruses (Shafer & Schapiro, 2008). However, several patients harbouring HIV-1 species containing the L74V point mutation while receiving a regimen containing ABC or ddI have been found to have minor variants containing K65R (Descamps *et al.*, 2006; Svarovskaia *et al.*, 2007). On the other hand, K65R and M184V mutations are primarily found in patients receiving the NRTI backbone TDF/3TC (Gallant *et al.*, 2004; Margot *et al.*, 2006) and less commonly ABC/3TC (Moyle *et al.*, 2005) or TDF/FTC (Gallant *et al.*, 2006; Smith *et al.*, 2008). L74V and M184V mutations primarily occur in patients receiving ABC/3TC or ddI/3TC/FTC NRTI backbones (Descamps *et al.*, 2006; Moyle *et al.*, 2005; Sosa *et al.*, 2005). In addition to the M184V, K65R and L74V mutations, which between them cause resistance to ABC, 3TC, FTC, TDF and ddI by the exclusion mechanism; there is a complex set of several mutations that involves Q151M causing multi-NRTI resistant (Figure 2 and Table 2). All of the amino acids involved in the NRTIs resistance by the exclusion mechanism are in the fingers or the palm of RT (Figure 2) and could potentially affect the binding of an incoming deoxy-nucleotide triphosphate (dNTP).

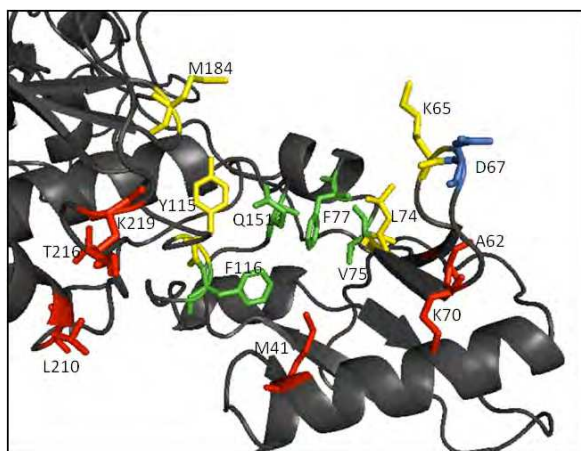


Fig. 2. Ribbon representation of the NRTI-binding site (palm and fingers of RT) showing residues that confer resistant when mutated as illustrated in Table 2. Red show amino acid residues that confer multi-NRTI resistance (all the FDA approved drugs). Green show residues that affect all the FDA approved NRTIs except for TDF. Mutations of blue residues are associated with TAMs. Yellow shows residues that confer resistance to certain NRTIs (Table 2) by the exclusion mechanism.

NRTI	TAMs (excision)						Non-TAMs (exclusion)					Multi-NRTI resistance mutations						
	M41	D67	K70 ^a	L210	T215	K219	K65	K70 ^a	L74	V75 ^b	Y115	M184	T69	Q151	A62	V75 ^b	F77	F116
3TC	-	-	-	-	-	-	RN	EG	-	-	-	VI	Ins	M	V	-	-	-
ABC	L	N	-	W	FY	-	RN	EG	VI	TM	F	VI	Ins	M	V	I	L	Y
D4T	L	N	R	W	FY	QE	RN	-	-	TM	-	-	Ins	M	V	I	L	Y
ddI	L	N	-	W	FY	-	RN	EG	-	TM	-	VI	Ins	M	V	I	L	Y
FTC	-	-	-	-	-	-	RN	EG	-	-	-	VI	Ins	M	V	-	-	-
TDF	L	N	-	W	FY	-	RN	EG	-	M	F	-	Ins	M	V	-	-	-
ZDV	L	N	R	W	FY	QE	-	-	-	-	-	-	Ins	M	V	I	L	Y

*Denotes position of consensus amino acids that confer resistance to NRTIs when mutated. Dashes (-) indicate that the respective NNRTIs are not affected by the mutation(s). All amino acids are denoted by the single letter annotation, except for "Ins", which stands for one or more amino acid insertions. Mutations in bold are associated with high levels of phenotypic resistance or reduced virologic response. ^aK70R occur in HIV-1 isolates from patients receiving thymidine analogs. ^bV75I occurs in combination with Q151M. Modified from (Shafer & Schapiro, 2008).

Table 2. Mutations in the RT gene associated with resistance to NRTIs

In contrast to the exclusion mechanism discussed above; the excision mechanism involves selective removal of the NRTI from the end of the viral DNA after it has been incorporated by RT (Arion *et al.*, 1998; Boyer *et al.*, 2001; Meyer *et al.*, 1999; Meyer *et al.*, 1998). This is ATP-dependent excision and polymerization run in reverse, where an AZT resistant RT efficiently incorporates AZT triphosphate but has the enhanced ability to remove the incorporated AZT-monophosphate from the 5'-end of the template strand (Arion *et al.*, 1998; Meyer *et al.*, 1998). The first and well studied example of excision mechanism involves a set of AZT resistant mutations such as M41L, D67N, K70R, L210W, T215F/Y and K219E (Figure 1 and Table 1). These AZT resistant mutations are also called thymidine-analog mutations (TAMs) or excision-enhancing mutations (EEMs). In addition to AZT, the TAMs decrease susceptibility to d4T and to a lesser extent ABC, ddI and TDF (Whitcomb *et al.*, 2003). TAMs are common in low income countries in which the fixed-dose combinations containing thymidine analogues are the mainstay of therapy (Ross *et al.*, 2001). TAMs are also common in HIV-1 isolates from patients who began therapy in the pre-highly active antiretroviral therapy (HAART) era (De Luca *et al.*, 2006; Rhee *et al.*, 2005). The mutations accumulate in two distinct but overlapping patterns (De Luca *et al.*, 2006; Gonzales *et al.*, 2003; Marcelin *et al.*, 2004b; Yahi *et al.*, 1999). The type I pattern includes the M41L, L210W and T15Y mutations, respectively (De Luca *et al.*, 2006; Miller *et al.*, 2004). Type II patterns includes D67N, K70R, T215F and K219Q/E (Figure 2). D67N point mutation also occurs commonly with type I TAMs (Cozzi-Lepri *et al.*, 2005; Rhee *et al.*, 2007). However, K70R and L210W rarely occur together (Yahi *et al.*, 2000). Type I TAMs cause higher levels of phenotypic and clinical resistance to the thymidine analogs and cross resistance to ABC, ddI and TDF than do the type II TAMs (Cozzi-Lepri *et al.*, 2005; De Luca *et al.*, 2007; Lanier *et al.*, 2004a; Miller *et al.*, 2004). The clinical significance of the type II TAMs is not well understood.

3.2 Point mutations associated with resistance to NNRTIs

The NNRTIs block RT activity allosterically by binding to a hydrophobic pocket close to but not contiguous with the polymerase active site (Figure 3). The NNRTI-binding pocket

(NNIBP), which exists only in bound RT (Kohlstaedt *et al.*, 1992a; Ren *et al.*, 1995a), consists of the following amino acid residues: L100; K101; K013; V106; T107; V108; V179, Y181; Y188; V189; G190; F227; W229; L234; Y318 and E138 (Figure 3). Nearly all the of the NNRTI resistance mutations are within the NNIBP or adjacent to residues in the pocket (Ren *et al.*, 2008; Ren & Stammers, 2008; Sarafianos *et al.*, 2004). The NNRTI resistant mutations are broadly classified as: (a) primary mutations that cause high-level resistance to one or more NNRTIs and are the first to develop during the therapy; (b) secondary mutations that usually occur in combination with primary NNRTI resistance mutations (c) minor non-polymorphic mutations that may occur alone or in combination with other NNRTI resistance mutations and (d) polymorphic accessory mutations that modulate the effects of other NNRTI resistance mutations (Shafer & Schapiro, 2008). There is low genetic barrier to NNRTI resistance. Only one or two mutations are required for high level resistance.

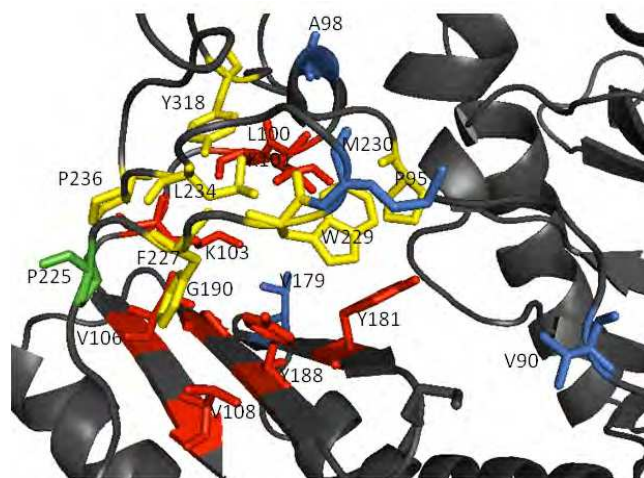


Fig. 3. Ribbon representation of the NNIBP showing the residues where NNRTI-resistance mutations occur. Red shows residues where primary point mutations which cause resistance to nearly all the current NNRTIs occur. Green shows a residue where a major secondary mutation which causes resistance to EFV occurs. Blue shows unique residues which confers resistance to ETR when mutated. Yellow shows residues where miscellaneous mutations that cause resistance to at least one of the NNRTI may occur.

The most frequently observed primary point mutations in patients treated with approved NNRTIs are K103N and Y181C (Table 3). Each of the primary NNRTI resistance mutations, namely, K103N/S; V106A/M; Y181C/I/V, Y188L/C/H and G190A/S/E (Figure 3 and Table 3) cause high level resistance to nevirapine (NVP) and variable resistance to efavirenz (EFV), ranging from about 2-fold for V106A and Y181C; 6-fold for G190A; 20-fold for K103N; and more than 50-fold for Y188L and G190S (Bachelier *et al.*, 2001; Rhee *et al.*, 2006b). Transient virologic responses to EFV-based salvage therapy regimen occur in some NNRTI-experienced patients but a sustained response is not common (Antinori *et al.*, 2002; Delaugerre *et al.*, 2001; Shulman *et al.*, 2000; Walmsley *et al.*, 2001). On the other hand, patients with any of the NNRTI resistance primary point mutations (Figure 3 and Table 3)

may benefit from etravirine (ETR) salvage therapy (Lazzarin *et al.*, 2007; Madruga *et al.*, 2007). However, point mutations at Y181 and to a lesser extent G190 compromise ETR response and may provide the foundation for the development of high-level ETR resistance (Lazzarin *et al.*, 2007; Madruga *et al.*, 2007). Major secondary and minor/miscellaneous mutations that usually occur in combination with one of the primary NNRTI resistance point mutations are depicted in Figure 3 and their respective effects on NNRTIs is summarized in Table 3.

Current understanding suggests that there are at least three classes of NNRTI-resistance mechanisms (Sarafianos *et al.*, 2009). The first mechanism involves loss or change of key hydrophobic amino acid residues in the NNIBP such as Y181, Y188 and F227 (Figure 3). Specific point mutations in one of these hydrophobic residues cause significant resistance through the loss of the aromatic ring interactions with NNRTIs (Das *et al.*, 1996; Kohlstaedt *et al.*, 1992a; Ren *et al.*, 1995b; Ren *et al.*, 2001; Ren *et al.*, 2004). This causes high levels of resistance to the first generation NNRTIs, which are relatively rigid (Sarafianos *et al.*, 2009). Second generation and more advanced NNRTI are designed with an inherent flexibility. This intrinsic flexibility called the wiggling and jiggling makes the second and subsequent generation NNRTIs to have compensatory interactions with RT that has mutations causing resistance to first generation NNRTIs (Das *et al.*, 2008; Das *et al.*, 2004). The second mechanism of NNRTI-resistance point mutations involves steric hindrance by amino acid residues in the central region of the NNIBP such as L100 and G190 (Figure 3). The L100I point mutation confers high-level resistance to all the current NNRTIs (Table 3) by changing the shape of the pocket (Ren *et al.*, 2004). In contrast G190A point mutation, which also confers high-level resistance to almost all the current NNRTIs (Table 3), causes a bulge (Sarafianos *et al.*, 2004). The third and last mechanism of NNRTI-resistance point mutation involves pocket entrance mutations at the rim of the NNIBP such as K103N and K101E (Figure 3). These point mutations cause resistance to first generation NNRTIs by interfering with the entry of NNRTIs into the NNIBP pocket (Hsiou *et al.*, 2001; Ren *et al.*, 2007). Second generation NNRTIs were designed to circumvent this problem. For instance, diarylpyrimidine NNRTIs such as ETR are able to inhibit HIV-1 isolates with K103N point mutation because they interact with the side chain of the mutated N103 residue (Das *et al.*, 2004; Janssen *et al.*, 2005).

NRTI	Primary mutations					Major secondary mutations					Minor and miscellaneous mutations				
	K103	V106	Y181	Y188	G190	L100	K101	P225	F227	M230	A98	V108	V179	P236	K238
*															
DLV	NS	AM	CIV	LHC	E	I	EP	-	C	L	G	I	DEF	L	NT
EFV	NS	AM	CIV	LHC	ASE	I	EP	H	C	L	G	I	DEF	-	NT
ETR	-	-	CIV	LHC	ASE	I	EP	-	C	L	G	-	DEF	-	-
NVP	NS	AM	CIV	LHC	ASE	I	EP	-	LC	L	G	I	DEF	-	NT

*Denotes position of consensus amino acids that confer resistance to NNRTIs when mutated. Dashes (-) indicate that the respective NNRTIs are not affected by the mutation(s). All amino acids are denoted by the single letter annotation. Mutations in bold are associated with high levels of phenotypic resistance or reduced virologic response. Modified from (Shafer & Schapiro, 2008).

Table 3. Mutations in the RT gene associated with resistance to NNRTIs

3.3 Point mutations associated with resistance to protease inhibitors

Resistance mutations in the protease genes are classified as major or minor (V. A. Johnson *et al.*, 2010). Major mutations are defined as those selected first in the presence of the protease inhibitor (PI) antiretroviral drug or those substantially reducing drug susceptibility. These mutations tend to be the primary contact residues for drug binding (Figure 4). Major PI resistance mutations that are of the most clinical significance occur at 17 largely non-polymorphic positions (Figure 4). Mutations at 13 of these 17 positions, including mutations at the substrate cleft positions L23, D30, V32, I47, G48, I50, V82 and I84; the flap positions M46 and I54; and the interior enzyme positions L76, N88 and L90 (Figure 4), reduce susceptibility to at least one PI (Table 4). Mutations at the other 4 positions (L24, L33, F53 and G73) are also important because they are common, non-polymorphic and cause resistance to several PI (Rhee *et al.*, 2006b).

Many major mutations reduce susceptibility to nelfinavir. In particular, L31I, D30N, M46I/L, G48V/M, I84V, N88D/S and L90M mutations are associated with high levels of phenotypic resistance to nelfinavir because they cause inferior virologic response to therapy relative to that obtainable with most other PI (Johnston *et al.*, 2004; Patick *et al.*, 1996; Vray *et al.*, 2003; B. Winters *et al.*, 2008). Similarly; I50L, I84 and N88S mutations are associated with high levels of phenotypic resistance to atazanavir/r (Colonno *et al.*, 2004; Pellegrin *et al.*, 2006; Rhee *et al.*, 2006b; Vermeiren *et al.*, 2007; Vora *et al.*, 2006; B. Winters *et al.*, 2008). G48V/M, I84V and L90M mutations are relative contraindications to the use of saquinavir/r (Marcelin *et al.*, 2004a; Marcelin *et al.*, 2007; Zolopa *et al.*, 1999). V32I, I47V/A, I54L/M and I84V mutations are relative contraindications to the use of fosamprenavir/r (Dandache *et al.*, 2007; Masquelier *et al.*, 2008; Pellegrin *et al.*, 2007; B. Winters *et al.*, 2008). V82A/F/T and I84V mutations are relative contraindications to the use of indinavir/r (Table 4). V47A and V82L/T major mutations are relative contraindications to the use of lopinavir/r (Dandache *et al.*, 2007; Friend *et al.*, 2004; Kagan *et al.*, 2005) and tipranavir/r (Baxter *et al.*, 2006). Table 4 lists the 17 major PI resistance mutations that are of clinical significance.



Fig. 4. Bound crystal structure of HIV-1 protease showing the residues where major PI-resistance mutations occur. (A) Mesh illustration of HIV-1 protease in bound state with saquinavir. (B) Illustration of amino acid residues (colour coded red) where PI-resistance point mutations occurs. (C) Zoomed-in illustration showing in detail residues (colour coded blue) where major PI-resistance mutations occur.

In contrast to the major PI resistance mutation discussed above; minor PI resistance mutations generally emerge later than major mutations and they do not have substantial effect on drug resistance phenotype by themselves. Instead, they improve replication of viruses containing major mutations (V. A. Johnson *et al.*, 2010). For instance, minor mutations at amino acid residues L10, K20, M36, L63 and A71 up-regulates protease processivity to compensate for the decreased fitness associated with the major PI resistance mutations (Hoffman *et al.*, 2003; Mammano *et al.*, 1998; Martinez-Picado *et al.*, 1999; Nijhuis *et al.*, 1999; van Maarseveen *et al.*, 2006). Residues L10, M36 and L63 are highly polymorphic. Baseline mutations at residues L10 and M36 are associated with an increased risk of virologic failure in patients receiving first generation PI such as nelfinavir (Perno *et al.*, 2001; Perno *et al.*, 2004). L10V and A71V/T minor mutations respectively occur in 5% and 10% of PI-naïve patients, and in much higher proportion of PI-experienced patients (Rhee *et al.*, 2003). In comparison, L10F/R and A71I/L minor mutations do not occur in the absence of PI therapy (Rhee *et al.*, 2003). Additional PI-selected mutations include the highly polymorphic mutations I13V, D60E, I62V, V77I and I93L as well as several uncommon mutations including V11I, E34Q, E35G, K43T, K45I, K55R, Q58E, T74P/A/S, V75I, N83D, P79A/S, I85V, L89V, T91S, Q92K and C95F (Ceccherini-Silberstein *et al.*, 2004; Parkin *et al.*, 2003; Rhee *et al.*, 2005; Svicher *et al.*, 2005; Vermeiren *et al.*, 2007; T. D. Wu *et al.*, 2003).

The mechanism of some PI resistance mutations seems subtype-specific. For example, the accessory PI resistance mutations I13V, K20I, M36I and I93L represent the consensus variant in one or more non-subtype B isolates (Rhee *et al.*, 2006a). Furthermore, although both D30N and L90M mutations occur in non-subtype B viruses during nelfinavir therapy, D30N point mutation occurs more commonly in subtype B viruses; while L90M occurs more commonly in subtypes C, F, G and circulating recombinant form-AE (CRF-AE) viruses (Abecasis *et al.*, 2005; Calazans *et al.*, 2005; Cane *et al.*, 2001; Grossman *et al.*, 2004; Sugiura *et al.*, 2002). The increased predilection for a certain subtype to develop L90M may relate to the presence of variants other than L, which is subtype B consensus, at position 89 (Abecasis *et al.*, 2005; Calazans *et al.*, 2005; Gonzalez *et al.*, 2004). Similarly, T74S, which is a polymorphism that occurs in 8% of subtype C sequences, but rarely in other subtypes, is associated with reduced susceptibility to nelfinavir (Deforche *et al.*, 2007; Deforche *et al.*, 2006; Rhee *et al.*, 2006b). Notwithstanding, with notable aforementioned exceptions, the genetic mechanisms of PI resistance are highly similar among different subtypes (Kantor *et al.*, 2005).

3.4 Point mutations associated with resistance to integrase inhibitors

The HIV-1 integrase catalyses the ligation of the viral 3'-OH end to the 5'-DNA of host chromosomal DNA through a process called the strand transfer (A. A. Johnson *et al.*, 2006; Pommier *et al.*, 2005). The current generation of clinically relevant INI drugs such as the recently USA Food and Drug Administration (FDA) approved raltegravir (FDA, 2007) and elvitegravir, currently undergoing phase III clinical trials (Gilead Sciences, 2008), preferentially inhibit strand transfer by binding to the target DNA site of the enzyme. Most INI resistance mutations are in the vicinity of the putative INI binding pocket (Figure 5). Some of the INI resistance mutations decrease susceptibility by themselves, whereas others compensate for the decrease fitness associated with other INI resistance mutations (Lataillade *et al.*, 2007). There is a high level of cross-resistance between raltegravir and elvitegravir, as well as between these INI and the first generation of strand-transfer

inhibitors, suggesting that the development of non cross-resistant INI will be challenging (Hombrouck *et al.*, 2008; Shimura *et al.*, 2008).

Common INI resistance point mutations are N155H and Q148H/R/K (Table 5), each of which can reduce raltegravir susceptibility by up to 25-fold (Cooper *et al.*, 2008). Higher levels of raltegravir resistance occur with accumulation of additional mutations. E92Q mutation and the two polymorphic mutations L74M and G163R generally occur with N155H, while G140A/S generally occurs with Q148H/R/K (Cooper *et al.*, 2008). Additional mutations (listed in Table 4) have been reported as being selected either *in vitro* or *in vivo* by raltegravir and include the non-polymorphic L74R, E138A/K, Y143R/C/H, N155S, H183P, Y226D/F/H, S230R, and D232N and the polymorphic mutations T97A and V151I (Delelis *et al.*, 2011; Malet *et al.*, 2008; Mouscadet *et al.*, 2009). For both raltegravir and elvitegravir, virologic failure has generally been accompanied by 100-fold or greater decreases in susceptibility and the development of two or more INI resistance mutations. Mutations at amino acid residues E92, F121, G140, Q148 and N155 are associated with more than 10-fold decrease in susceptibility to both INI, whereas mutations at amino acid residues T66 and S147 are associated with marked decreases in susceptibility only to elvitegravir (Figure 5 and Table 5).

PI	Major mutations																
	L23	L24	D30	V32	L33	M46	I47	G48	I50	F53	I54	G73	L76	V82	I84	N88	L90
ATVr	-	I	-	-	F	IL	V	VM	L	<u>L</u>	VTALM	ST	-	ATFS	VAC	DS	M
DRVr	-	-	-	I	F	-	VA	-	V	-	LM	ST	V	-	VAC	-	M
FPVr	-	-	-	I	F	IL	VA	-	V	-	VTALM	ST	V	ATFS	VAC	-	M
INDVr	-	I	-	V	-	IL	V	-	-	L	VTALM	ST	V	AFTS	VAC	S	M
LPVr	-	I	-	I	F	IL	VA	VM	V	-	VTALM	-	V	AFTS	VAC	-	M
NFV	I	I	N	-	F	IL	V	VM	-	L	VTALM	ST	-	AFTS	VAC	DS	M
SQVr	-	I	-	-	-	-	-	VM	-	L	VTALM	ST	-	AT	VAC	S	M
TPVr	-	-	-	I	F	IL	V	-	-	-	VAM	-	-	ATFSL	VAC	-	M

*Denotes position of consensus amino acids that confer resistance to PI when mutated. Dashes (-) indicate that the respective PI are not affected by the mutation(s). All amino acids are denoted by the single letter annotation. Mutations in **bold** are associated with high levels of phenotypic resistance or reduced virologic response. Mutations in **bold underline** are relative contraindications to the use of specific PI. ATVr (atazanavir/r); DRVr (darunavir/r); FPVr (fosamprenavir); IDVr (indinavir/r); NFV (nelfinavir); SQVr (saquinavir/r); TPVr (tipranavir/r). Modified from (Shafer & Schapiro, 2008).

Table 4. Mutations in the protease gene associated with major resistance to PI

INI	Mutations											
	T66	E92	F121	E138	G140	Y143	S147	Q148	S153	N155	E157	R263
Raltegravir	-	Q	Y	AK	AS	CHR	G	HRK	-	HS	Q	-
Elvitegravir	I	Q	Y	AK	AS	-	G	HRK	Y	HS	Q	K

*Denotes position of consensus amino acids that confer resistance to INI when mutated. Dashes (-) indicate that the respective INI are not affected by the mutation(s). All amino acids are denoted by the single letter annotation. Mutations in **bold** are associated with more than 5-10 fold phenotypic resistance or reduced virologic response. Modified from (Shafer & Schapiro, 2008).

Table 5. Mutations in the integrase gene associated with resistance to INI

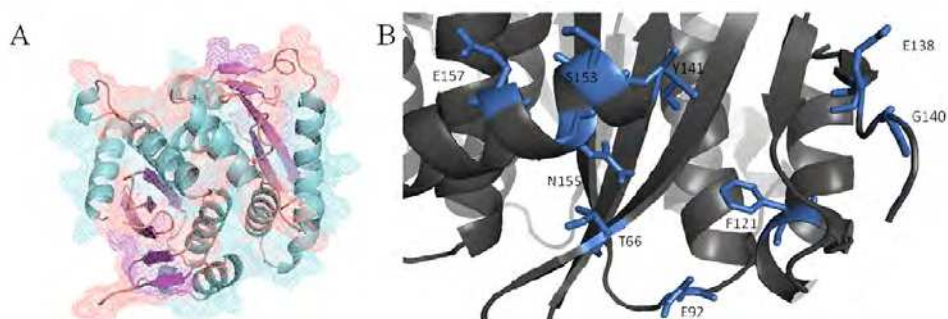


Fig. 5. Crystal structure of HIV-1 integrase showing the residues where major INI-resistance mutations occur. (A) Mesh illustration of bound HIV-1 integrase. (B) Illustration of amino acid residues where INI-resistance point mutations occurs.

3.5 Point mutations associated with resistance to fusion inhibitors

The HIV-1 gp41 mediates the fusion between the virus and host cell membrane. Fusion inhibitors act by a dominant negative mechanism of action, preventing formation of the six-helix bundle that is the driving force for the fusion process. Enfuvirtide, also known as T20 (Kilby *et al.*, 1998; Wild *et al.*, 1994), is the only FDA approved fusion inhibitor to date. Enfuvirtide has antiviral activity similar to most other classes of antiretroviral drugs discussed above; however resistance develop rapidly in patients receiving the drug for salvage therapy who do not receive a sufficient number of RTI, PI and INI drugs such as efavirenz, lopinavir/r and raltegravir, respectively. The emergence of enfuvirtide resistant strains followed by virologic rebound has been observed in some patients within two to four weeks (Cabrera *et al.*, 2006; Lu *et al.*, 2006).

Mutations in gp41 codons 36 to 45 (Figure 6), the region to which enfuvirtide binds, are primarily responsible for resistance to the drug (Marcelin *et al.*, 2004c; Melby *et al.*, 2006; Menzo *et al.*, 2004; Mink *et al.*, 2005; Sista *et al.*, 2004; Su *et al.*, 2006). The most common and potent T20-resistance mutations in this region are G36D/E, V38E/A, Q40H and N43D/K/S (Table 6). A single point mutation is generally associated with about 10-fold decreased susceptibility; whereas double point mutations can decrease susceptibility by more than 100-fold (Charpentier *et al.*, 2011; Su *et al.*, 2006; Xu *et al.*, 2005). The replication fitness of recombinant viruses carrying the enfuvirtide-resistant mutations is relatively poor (Lu *et al.*, 2004) and the mutant viruses rapidly revert to wild type phenotype in patients who discontinue the drug (Deeks *et al.*, 2007). Some enfuvirtide-resistant mutations, particularly V38A/E mutations, are associated with significant CD4 count increases (Aquaro *et al.*, 2006), presumably because mutations at residue V38 decrease HIV-1 replication or render the virus more susceptibility to a subset of neutralizing antibodies that target fusion intermediates (Reeves *et al.*, 2005).

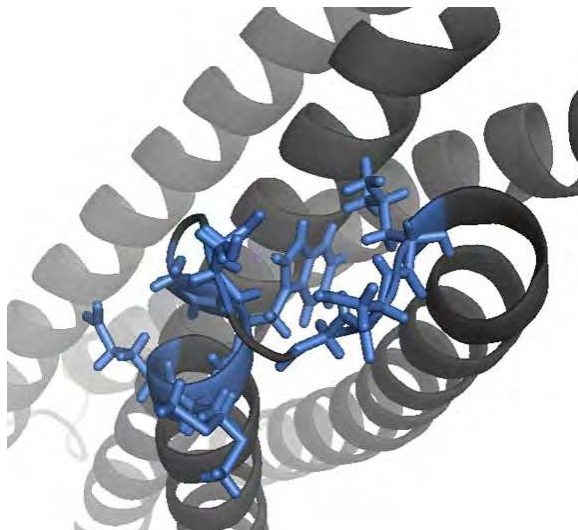


Fig. 6. Ribbon representation of HIV-1 heptad repeat fusogenic domain showing point mutations (colour coded blue) that cause resistance to fusion inhibitors.

Fusion inhibitor	Mutations								
*	G36	I37	V38	Q39	Q40	N42	N43	L44	L45
Enfuvirtide	DEVS	V	EAMG	R	H	T	DKS	M	M

*Denotes position of consensus amino acids that confer resistance to enfuvirtide when mutated. All amino acids are denoted by the single letter annotation. Mutations in bold are associated with more 10-fold phenotypic resistance or reduced virologic response in most clinical isolates. Modified from (Shafer & Schapiro, 2008).

Table 6. Mutations in the gp41 *env* gene associated with resistance to fusion inhibitors

3.6 Point mutations associated with resistance to attachment inhibitors

Despite the important role gp120 plays in attachment of HIV-1 to the CD4 receptor and CCR5/CXCR4; maraviroc, which is a CCR5 antagonist, is the only FDA approved attachment inhibitor in clinical use to date. While gp120 binds to the N-terminus and the second extracellular loop region of CCR5 (Hartley *et al.*, 2005), site-directed mutagenesis study suggests that small molecule attachment inhibitors such as maraviroc bind to a CCR5 pocket formed by the transmembrane helices (Dragic *et al.*, 2000). Thus, although gp120 and maraviroc do not bind to the same site of the CCR5 coreceptor, maraviroc allosterically inhibit gp120 binding to the CCR5 coreceptor. Point mutations in gp120 that allow the virus to bind to the maraviroc-bound form of CCR5 in the drug insensitive manner have been described (Westby *et al.*, 2007). Most of these mutations are found in the V3 loop of gp120 (Figure 7A), which determines HIV-1 tropism (discussed in the next section). One study showed that reverse mutations at positions 316 and 323 in the V3 loop of maraviroc-resistant HIV-1 variants to their original sequence restored wild-type susceptibility to maraviroc,

while reversion of either mutation resulted in a partially sensitive virus with reduced maximal inhibition (Westby *et al.*, 2007). In another recent study maraviroc-resistant variants were isolated from the V3 loop library virus (HIV-1(V3lib)) containing the T199K and T275M plus 5 mutations in the V3 loop, namely I304V; F312W; T314A; E317D; I318V (Yuan *et al.*, 2011). Furthermore, the same study showed that the profile of HIV-1(JR-FL) pseudotype containing I304V/F312W/T314A/E317D/I318V (Figure 7A) in the V3 loop alone revealed a typical non-competitive resistance to maraviroc (Yuan *et al.*, 2011). Generally, amino acids mutations in the V3 loop may include known polymorphism as well as novel substitutions, insertions, and deletions. Further complicating the genetic basis of maraviroc or CCR5 inhibitor resistance is the observation that the same inhibitor may select for different mutations in different HIV-1 isolates (Westby *et al.*, 2007). The mutations observed in maraviroc-resistant strains isolated from patients are variable and differed for each HIV-1 isolate (Seclen *et al.*, 2011; Tilton *et al.*, 2011).

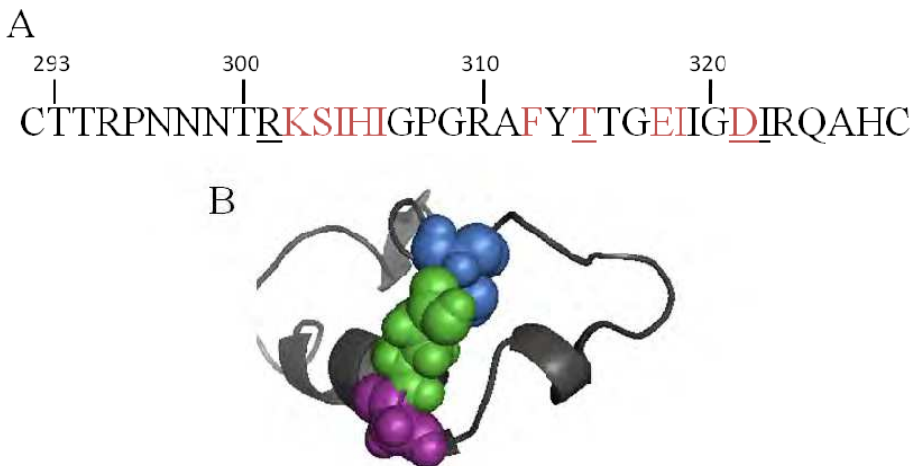


Fig. 7. Illustration of amino acid residues in the V3 loop important for HIV-1 tropism and resistance to maraviroc. (A) The sequence of the V3 variable loop indicating the sites of unique X4 substitution sites when compared to R5 clones (underlined). The red residues indicate the positions where maraviroc resistance mutations occur. Together with T199K and T275M, these mutations confer complete resistance to maraviroc. (B) Ribbon diagram of gp120 V3 variable loop highlighting amino acid residues essential for co-receptor usage. X4 tropism is determined by positively charged residues at position 11, 24 and 25 (colour coded blue, green and magenta respectively). R5 tropism is determined by a negative or neutral residue at any of the three positions.

4. Point mutations associated with HIV-1 tropism, transmission dynamics and AIDS pathogenesis

The identification of CCR5 and CXCR4 chemokine receptors as having critical roles in the cellular entry of HIV-1 allowed development of a more precise system for defining the viral tropism. Prototype T-cell tropic strains use CXCR4, macrophage-tropic strains use CCR5,

and dual-tropic strains use both CXCR4 and CCR5 chemokine receptors, culminating in the currently used nomenclature X4, R5 and R5X4 strains, respectively (Berger et al., 1998). The phenotype of HIV-1 that is transmitted *in vivo* is R5 even when the contaminating virus is a mixture of R5, R5X4 and X4. The reason for this selection of viral phenotype during transmission is not well understood. The viraemia present during primary infection consists of oligoclonal R5 virus, and this virus type generally persists during the asymptomatic phase of the infection until the onset of AIDS. Viral transmission and disease progression, collectively called AIDS pathogenesis, corresponds to evolution of coreceptor phenotype. In about half of all HIV-1 infections there is viral evolution towards dual (R5X4) tropism and CXCR4 usage that is prognostic for accelerated progression to AIDS (Collman et al., 1992; Doranz et al., 1996). Thus when the phenotype switch occurs, there is an initial evolution from CCR5 using viruses to those that can use both CCR5 and CXCR4 (Connor et al., 1997). While evolution towards usage of the CXCR4 coreceptor and emergence of X4 viruses coincides with a decline in CD4⁺ T cells and the onset of AIDS, the dominant theory is that X4 viruses are opportunistic infections that can only appear once the CD4⁺ T cells have dropped below a certain threshold level. Notwithstanding, in another 50% of individuals infected with HIV-1 there is no viral evolution towards CXCR4 usage or emergence of X4 viruses; R5 viruses persists from primary infection until one succumbs to AIDS. This suggests that R5 viruses are necessary and sufficient to cause AIDS.

The main determinants for coreceptor tropism resides in the V3 loop (Figure 7), although mutations outside of the V3 loop may also influence tropism, either in combination or independently of the V3 mutations (Hartley et al., 2005; W. Huang et al., 2008; Pastore et al., 2007; Pastore et al., 2006). Very few mutations in the V3 loop are sufficient for switching coreceptor use from CCR5 to CXCR4 in most HIV-1 isolates, but in some isolates additional mutations in V1/V2 (Pastore et al., 2006) and gp41 (W. Huang et al., 2008) are observed during coreceptor switching. The presence of positively charged amino acids at positions 11, 24 and 25 in the V3 loop, combined with other V3 sequence characteristics (Figure 7), have specificity of about 90% and sensitivity of 70- 80% for predicting X4 tropism (Hartley et al., 2005; Jensen & van 't Wout, 2003; Sing et al., 2007). However, the number and type of mutations by which an R5 virus switches to X4 is complex and depends on the sequence of the baseline R5 virus (Low et al., 2007; Moncunill et al., 2008; Pastore et al., 2007; Pastore et al., 2006). The frequency and genetic basis for tropism switch is different for different HIV-1 subtypes (Hartley et al., 2005; W. Huang et al., 2007). While differential use of CXCR4 or CCR5 by HIV-1 strains has been observed, the striking differences seen appear to be in how R5 and R5X4 viruses utilize the CCR5 coreceptor. Generally, R5-tropic viruses are particularly well adapted for CCR5 use (Choe et al., 1996; Deng et al., 1996; Dragic et al., 1996), and can tolerate deletion or substitution of the CCR5 amino-terminal domain (Howard et al., 1999; Z. Wang et al., 1999). R5X4 viruses, however, are much more sensitive to mutations in CCR5, particularly in the amino terminal domain (Doranz et al., 1997). Thus, there is a cost associated with the benefit to use dual chemokine receptors as exhibited by R5X4 tropic strains that have more stringent requirements for CCR5 usage (Rana et al., 1997).

Host genetic factors can also influence HIV-1 tropism and transmission. Cohorts of HIV-1 infected individuals have been established based on specific clinical, virological and immunological criteria. One type of cohort contains individuals selected after frequent

exposure to HIV-1 without seroconversion. A mutation in the CCR5 gene has been frequently found in this type of cohort (Zimmerman *et al.*, 1997). This mutation is a deletion of 32 base pairs ($\Delta 32$), resulting in a defective CCR5 receptor. CD4⁺ T cells and macrophages carrying the $\Delta 32$ mutation are resistant to infection with R5 viruses. Homozygous individuals carrying the mutation on both alleles (1% of Caucasians) are almost completely refractory to infection with R5 HIV-1 strains (Samson *et al.*, 1996; Zimmerman *et al.*, 1997). Very rare examples of infection of these individuals have been reported (O'Brien *et al.*, 1997; Theodorou *et al.*, 1997). In these cases the transmitted R5 viruses appear to use CXCR4. Individuals heterozygous for the $\Delta 32$ mutation are not protected from HIV-1 infection, but have slower rate of progression to AIDS. This is because the mutated form of the protein acts as a dominant negative mutant and prevents most of the wild-type CCR5 protein from reaching the cell surface. However, HIV-1 can evolve adaptive mutations on the V3 loop of gp120 that allows it to use defective or mutant CCR5 co-receptor. For instance, it has been shown that a N300Y adaptive point mutation on the V3 loop of gp120 enhances binding of HIV-1 to the mutant CCR5 co-receptor (Platt *et al.*, 2001). A follow-up study from the same group has shown that in addition to the adaptive mutation on the V3 loop of gp120, an S193N point mutation on the V2 stem and loss of N-linked oligosaccharide from position N403 of gp120 help HIV-1 to efficiently use defective CCR5 co-receptor that has a badly damaged amino terminus (Platt *et al.*, 2005). Taken together, these studies have important implications for understanding HIV-1 transmission dynamics and AIDS pathogenesis.

AIDS pathogenesis is multifactorial; it is a mixture of host and pathogen genetics combined with factors such as the immune response and viral adaptation. Viral adaptation include reverting and compensatory mutations such as the recently described T242N point mutation on the *gag* gene associated with progression to AIDS in South African patients (K. H. Huang *et al.*, 2011). However in general, progression from acute infection is often accompanied by depletion of the CD4⁺ lymphocytes and this depletion is a major component of the eventual failure of the immune system and hence AIDS pathogenesis. Apoptosis is one mechanism that contributes to depletion of HIV-1 infected and uninfected cells, deterioration of the immune system and progression to AIDS, respectively (Cotton *et al.*, 1997; Gougeon & Montagnier, 1993; Herbein *et al.*, 1998; Joshi *et al.*, 2011; C. J. Li *et al.*, 1995; Zhu *et al.*, 2011). HIV-induced apoptosis is triggered by a number of HIV-1 proteins, notably the envelope protein (Herbein *et al.*, 1998; Ishikawa *et al.*, 1998; Joshi *et al.*, 2011; Micoli *et al.*, 2006; Micoli *et al.*, 2000). Four point mutations (A835W, A838W, A838I, and I842R) in the cytoplasm domain of the gp41 subunit of the envelope protein reduces HIV-induced apoptosis of target cells (Micoli *et al.*, 2006). A recent study has shown that induction of bystander apoptosis in CCR5 expressing cells requires the fusogenic activity of gp41; and that V2E and V38A/E point mutation in the second amino acid and the heptad repeat 1 region of gp41 respectively, abrogated fusion activity and hence apoptosis induced by an R5 virus (Joshi *et al.*, 2011). In an earlier study, the same group showed that a V513E point mutation in the fusion domain of gp41 resulted in a fusion-defective envelope protein that failed to induce apoptosis and that a G547D mutation in the gp41 N-terminal helix also reduced cell fusion capacity and apoptosis induced by an X4 virus (Garg *et al.*, 2007).

5. Point mutations associated with evasion of antibody mediated immune response

Point mutations on HIV-1 envelope protein do not only influence HIV-1 tropism and disease progression, but they also help the virus to evade the antibody mediated immune response. The HIV-1 surface gp120 and the trans-membrane gp41 can elicit both virus-neutralizing and non-neutralizing antibodies during natural infection. Antibodies that lack neutralizing activity are often directed against the gp120 regions that are occluded on the assembled trimer, and which are exposed only upon shedding (Moore & Sodroski, 1996). Neutralizing antibodies, by contrast must access the functional envelope glycoprotein complex and typically recognize conserved or variable epitopes near the receptor binding sites (Posner *et al.*, 1991; Sattentau & Moore, 1995; Trkola *et al.*, 1996a; L. Wu *et al.*, 1996). Neutralising antibodies are also elicited by the membrane proximal external region (MPER) of gp41 (Frey *et al.*, 2008; J. Wang *et al.*, 2011; Zwick *et al.*, 2001). The production and characterization of monoclonal antibodies of human and rodent origin have allowed a precise determination of the neutralization epitopes present on the HIV-1 gp120 (Moore & Ho, 1995; Poignard *et al.*, 1996).

Using epitope maps in conjunction with the X-ray crystal structure of gp120 complexed with CD4 and a neutralizing monoclonal antibody (Kwong *et al.*, 1998b), spatial organization of conserved neutralization epitopes on gp120 was determined (Wyatt *et al.*, 1998). More broadly neutralizing antibodies recognize discontinuous, conserved epitopes in three regions of the gp120 (Figure 8). In HIV-1 infected individuals, the most abundant of these are directed against the CD4 binding site (CD4bs), and block gp120-CD4 interaction (Euler *et al.*, 2011; Ho *et al.*, 1991; Y. Li *et al.*, 2011; Posner *et al.*, 1991; Walker *et al.*, 2009; X. Wu *et al.*, 2010; Zhou *et al.*, 2011). Less common are antibodies against epitopes induced or exposed upon CD4 binding (CD4i) (Thali *et al.*, 1993). Both CD4i and V3 antibodies disrupt the binding of gp120-CD4 complexes to chemokine receptors (Trkola *et al.*, 1996a; L. Wu *et al.*, 1996). Notwithstanding, none of the CD4i antibodies discovered to date such as 17b, 48d or X5 are broadly neutralizing. A third gp120 neutralization epitope is defined by a unique monoclonal antibody, 2G12 (Trkola *et al.*, 1996b), which does not efficiently block receptor binding (Trkola *et al.*, 1996a). Table 6 lists broadly neutralising monoclonal antibodies elicited by the HIV-1 gp120 and gp41 envelope proteins. Although high-titer antibodies are sustained in infected individuals throughout the course of infection, these antibodies have very poor neutralizing activity against autologous as well as representative primary HIV-1 isolates (Montefiori *et al.*, 1996; Moog *et al.*, 1997). This observation suggests that neutralizing antibodies that arise in response to HIV-1 infection may not be critical in limiting viral replication.

The escape of HIV-1 from effects of neutralizing antibodies includes mutations in gp120 and/or gp41. One of the seminal studies showed that A582T point mutation in the HIV-1 envelope gene made a laboratory adapted HIV-1 strain called HXB2 to be resistant to HIV-1 neutralizing human serum (Reitz *et al.*, 1988). Another study published ten years later, showed that HIV-1 MN molecular clones which had 294N/K mutation before the V3 loop, 307N/I mutation proximal to the V3 loop, 327I/K mutation within V3 and 336N/I mutation after the V3 loop were resistant to human serum containing neutralizing antibodies directed at the immunodominant V3 loop of gp120 (Park *et al.*, 1998). Point mutations in a highly conserved structural motif within the intracytoplasmic tail of gp41 rendered HIV-1 molecular clones resistant to broadly neutralizing polyclonal human serum antibodies (Kalia *et al.*, 2005).

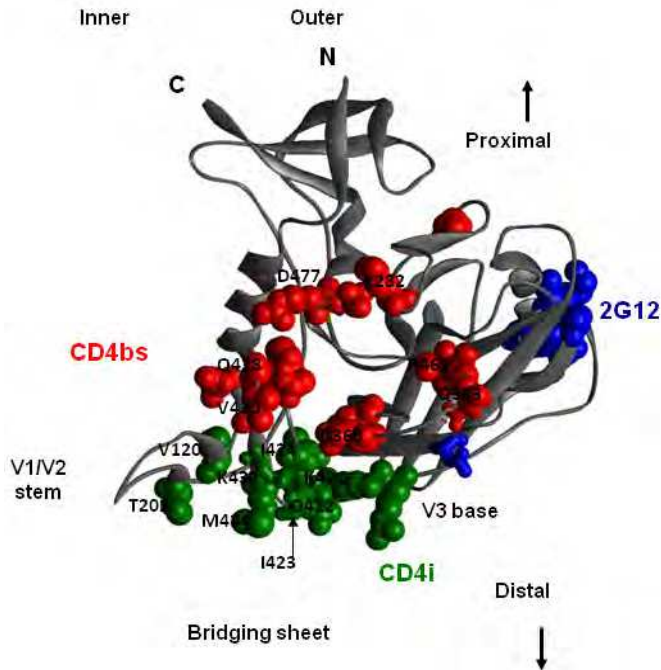


Fig. 8. Ribbon diagram illustrating epitopes of the gp120 core, highlighting some amino acid residues that interact with broadly neutralizing antibodies. The CD4bs (red) and the CD4i co-receptor-binding surface (green) as well as residues implicated in 2G12 binding (blue) are depicted.

In a clinically relevant study, paediatric HIV-1 subtype C clinical isolates with K665S/R/N point mutation were found to be resistant to a 2F5 broadly neutralizing monoclonal antibody (Gray *et al.*, 2006). This study confirmed earlier findings that the amino acid residue K665 is crucial for neutralization by 2F5 (Binley *et al.*, 2004). In the same study, the Lynn Morris' group found out that all the paediatric HIV-1 subtype C clinical isolates analysed had a conserved 4E10 epitope (W672, F673, W680) consistent with their phenotypic sensitivity to the 4E10 monoclonal antibody (Gray *et al.*, 2006). 4E10 recognizes an epitope containing the sequence NWF(D/N)IT (Zwick *et al.*, 2001). Mutagenesis studies have shown that the amino acid residues W672, F673 and W680 are indispensable for recognition by 4E10 (Zwick *et al.*, 2005). Indeed, a recently published study describing four subjects infected with viruses carrying rare MPER polymorphisms associated with resistance to 4E10 neutralization has shown that three subjects had W680 polymorphism, including a W680G point mutation (Nakamura *et al.*, 2011). The study demonstrated that W680G point mutation was necessary to confer 4E10 resistant phenotype (Nakamura *et al.*, 2011). In another subject a W680R point mutation caused variable resistant to 4E10 (Nakamura *et al.*, 2011). A fourth subject possessed a F673L point mutation also associated with 4E10 resistance (Nakamura *et al.*, 2011). Earlier, an independent study conducted in South Africa also showed that an HIV-1 subtype C virus isolated from a 7-year-old

perinatally infected child with F673L point mutation was resistant to 4E10 (Gray et al., 2008). These case studies are examples of the challenges presented by point mutations in controlling HIV-1 infection by antibodies. Notwithstanding, recent discovery of additional and more potent broadly neutralizing antibodies such as VRC01 (X. Wu *et al.*, 2010), PG9 and PG16 (Walker et al., 2009) is encouraging and boosts the impetus for HIV-1 vaccine design that may elicit broadly neutralizing antibodies (Burton & Weiss, 2010).

Epitopes	Example of mAb	Mechanism of neutralization	Properties	Selected references
CD4 binding site(CD4bs)	IgGb12, VRC01, HJ16, PG9 and PG16	Interfere with gp120-CD4 binding	CD4bs antibodies compete with CD4 and with antibodies against CD4i epitope. PG9 and PG16 bind to trimer specific glycosylated epitope on the V1/V2 and V3 region.	(Corti <i>et al.</i> , 2010; Euler <i>et al.</i> , 2011; Ho <i>et al.</i> , 1991; Y. Li <i>et al.</i> , 2011; Pancera <i>et al.</i> , 2010; Posner <i>et al.</i> , 1991; Walker <i>et al.</i> , 2009; X. Wu <i>et al.</i> , 2010; Zhou <i>et al.</i> , 2011)
Glycans	2G12	Interfere with binding of gp120 to CCR5 chemokine receptor and DC-SIGN. Also prevent loss of CD4+ T cells.	Antibody binding is dependent upon proper N-linked glycosylation. Recognize a mannose rich epitope on the “silent” face of gp120	(Binley <i>et al.</i> , 2006; Calarese <i>et al.</i> , 2003; Luo <i>et al.</i> , 2011; Scanlan <i>et al.</i> , 2003; Trkola <i>et al.</i> , 1996b)
MPER of gp41	4E10, 2F5	Interfere with HIV-1 fusion and induces shedding of gp120.	Both antibodies bind few crucial residues in the MPER of gp41. HIV-1 2F5 binds both native and fusion-intermediate conformations while 4E10 recognize a predominantly linear and relatively conserved epitope.	(Muster <i>et al.</i> , 1994; Muster <i>et al.</i> , 1993; Ruprecht <i>et al.</i> , 2011; Zwick <i>et al.</i> , 2005; Zwick <i>et al.</i> , 2001)

Table 8. Broadly neutralizing antibodies that bind gp120 and gp41 HIV-1 envelope proteins

7. Point mutations and anti-gp120 aptamers

Aptamers are synthetic oligonucleotide ligands that can be isolated *in vitro* against diverse targets including HIV-1 gp120 (Khati *et al.*, 2003). Aptamers assume a defined three-dimensional structure and generally bind functional sites on their respective targets. They possess the molecular recognition properties of monoclonal antibodies in terms of their high affinity and specificity (Khati, 2010). Unlike antibodies, aptamers can fold properly and retain activity in the intracellular environment. However, the majority of aptamers with potential therapeutic utility selected to date target extracellular proteins (Chen *et al.*, 2003; Eyetech Study Group, 2002, 2003; Green *et al.*, 1995; Kim *et al.*, 2003; Liu *et al.*, 2009; Lupold *et*

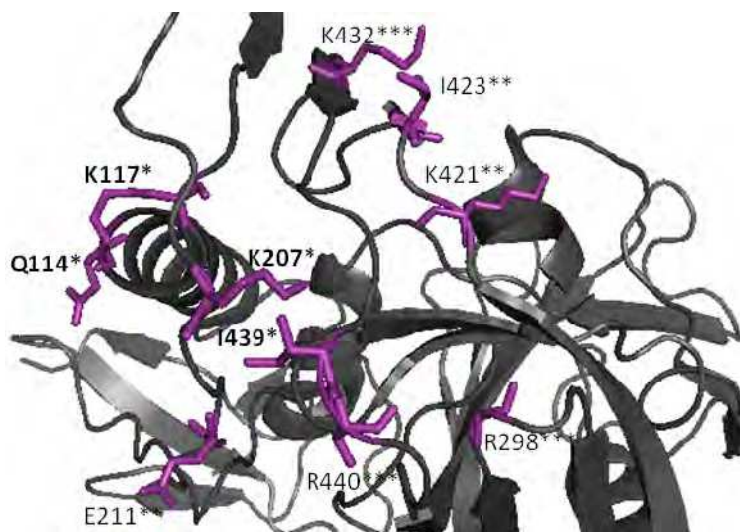


Fig. 9. A ribbon diagram of gp120 highlighting amino acid residues that interact with B40 aptamer. Residues in **bold** and single asterisk are highly conserved among all HIV-1 isolates and significantly reduced binding of the aptamer by more than 10-fold when experimentally mutated to alanine, respectively (Joubert *et al.*, 2010). Those residues with double or triple asterisk are moderately and significantly variable, respectively, in all HIV isolates and while they make contact with the aptamer they did not significantly affect binding of the aptamer when experimentally mutated to alanine, respectively.

al., 2002; Mi *et al.*, 2009; Nobile *et al.*, 1998; Ruckman *et al.*, 1998; Rusconi *et al.*, 2004; Rusconi *et al.*, 2002; Vinores, 2003; White *et al.*, 2003).

Extracellular therapeutic targets such as HIV-1 gp120 have the advantage of ready access to aptamer intervention without the need for enabling access to cells. The aptamers against HIV-1 gp120 bound the protein with high affinity, high specificity and neutralized a broad range of R5 HIV-1 clinical isolates (Khati *et al.*, 2003). In a recent review, these RNA aptamers were reported to have the most potent *in vitro* antiviral efficacy of all HIV-1 entry inhibitors described to date (Held *et al.*, 2006), including antibodies. These aptamers prevented entry and suppressed viral replication in cultured human peripheral blood mononuclear cells (PBMC) by up to 10,000-fold (Khati *et al.*, 2003). While the emergence of HIV-1 escape mutants and drug resistance variants appears to be inevitable, our group and collaborators have recently shown that one extensively studied aptamer called B40 penetrate the highly variable exterior surfaces of gp120 and bind the conserved core at the heart of the CCR5-binding site (Cohen *et al.*, 2008; Dey *et al.*, 2005; Joubert *et al.*, 2010), which the virus may be unable to mutate without compromising its fitness. Four amino acids (Q114, K117, K207, and I439) of gp120 (Figure 9) that weakened binding of gp120 to B40 aptamer by at least 10-fold when experimentally mutated to alanine (Joubert *et al.*, 2010) are naturally conserved among all HIV-1 isolates (Kwong *et al.*, 1998a). This suggests that B40 makes direct contact with at least four conserved core residues on gp120 within the CCR5-binding site, which the virus cannot afford to mutate without losing selective advantage. These data

bode well for the future development of aptamers such as B40 as an entry inhibitor drug, which will hopefully help circumvent HIV-1 point mutations and delay or diminish drug resistance. In order to maximally suppress or eradicate the virus; the aptamer or any other anti-HIV molecule in development should be used in combination with current antiretroviral drugs and the envisaged vaccines currently developed to elicit broadly neutralising antibodies.

8. References

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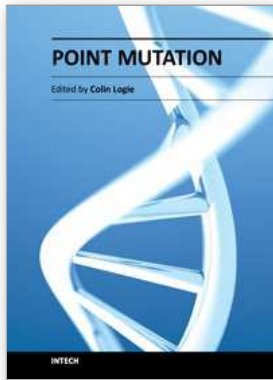
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Point Mutation

Edited by Dr Colin Logie

ISBN 978-953-51-0331-8

Hard cover, 352 pages

Publisher InTech

Published online 21, March, 2012

Published in print edition March, 2012

This book concerns the signatures left behind in chromosomes by the forces that drive DNA code evolution in the form of DNA nucleotide substitutions. Since the genetic code predetermines the molecular basis of life, it could have been about any aspect of biology. As it happens, it is largely about recent adaptation of pathogens and their human host. Nine chapters are medically oriented, two are bioinformatics-oriented and one is technological, describing the state of the art in synthetic point mutagenesis. What stands out in this book is the increasing rate at which DNA data has been amassed in the course of the past decade and how knowledge in this vibrant research field is currently being translated in the medical world.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Makobetsa Khati and Laura Millroy (2012). Point Mutations Associated with HIV-1 Drug Resistance, Evasion of the Immune Response and AIDS Pathogenesis, Point Mutation, Dr Colin Logie (Ed.), ISBN: 978-953-51-0331-8, InTech, Available from: <http://www.intechopen.com/books/point-mutation/point-mutations-associated-with-hiv-1-drug-resistance-evasion-of-the-immune-response-and-aids-pathog>

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