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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

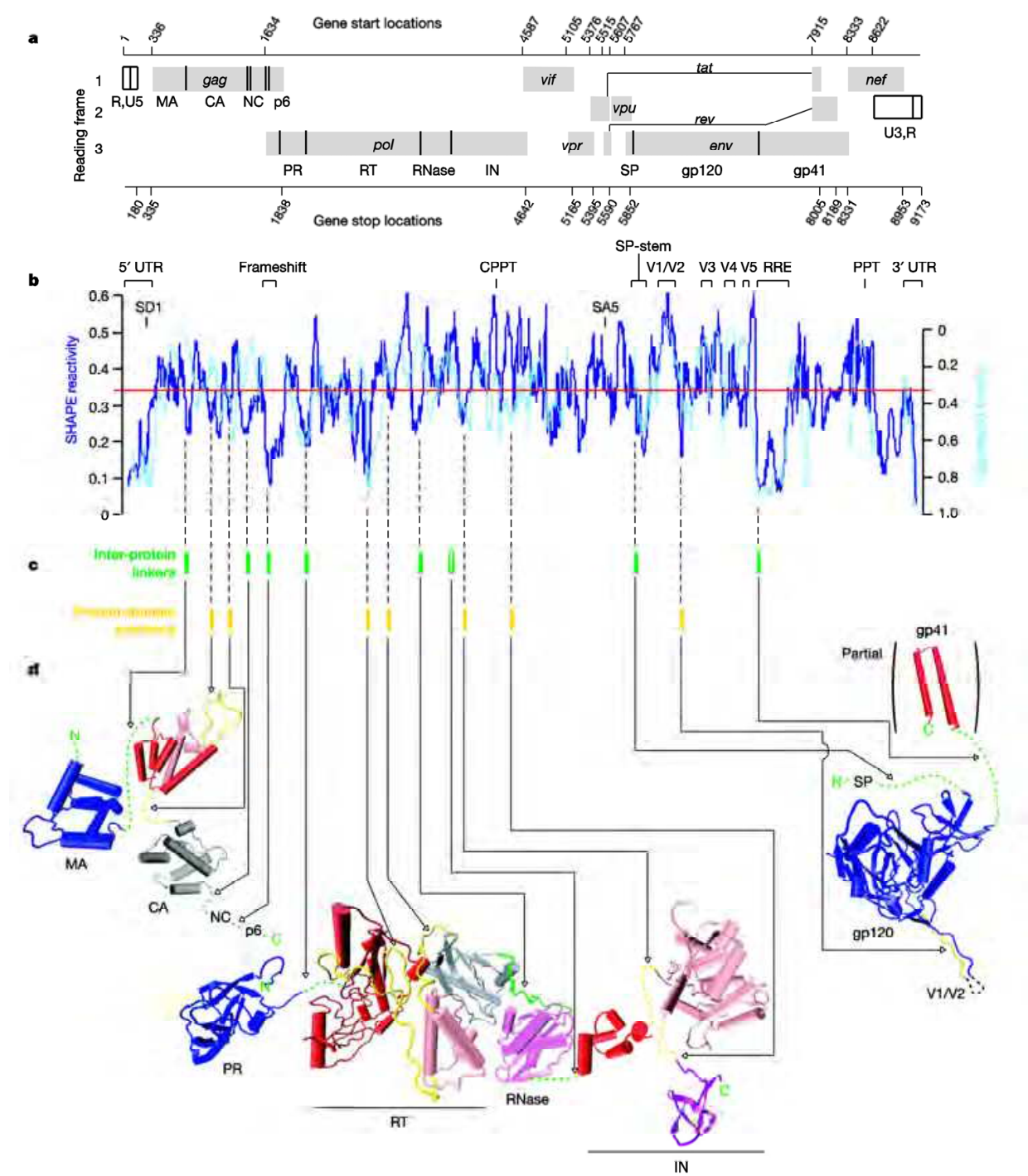


Fig. 1. Organization and function of the HIV-1 genome in relation to the proteins coded. (a) Protein coding region are shown as grey boxes. (b) Comparison of the pairing probability of nucleotides in the overall HIV-1 structure using 2'-hydroxyl acylation analysed by primer extension (SHAPE) reactivity. (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).

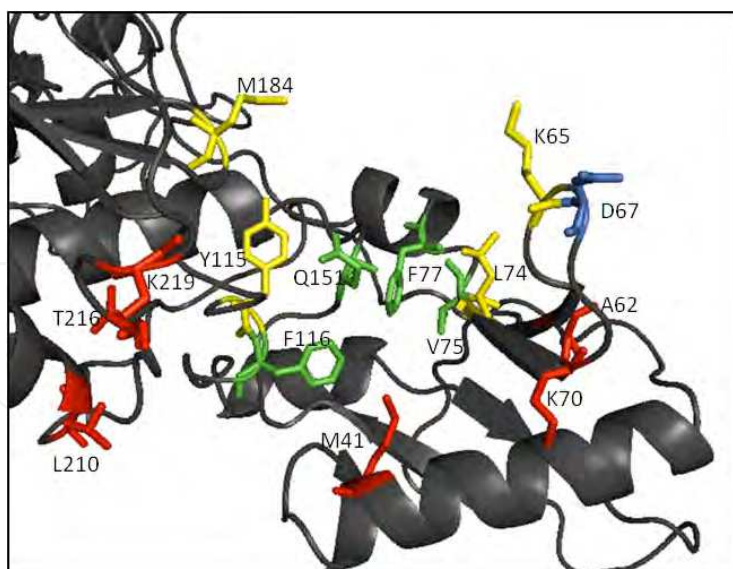


Fig. 2. Ribbon representation of the NRTI-binding site (palm and fingers of RT) showing residues that confer resistant when mutated as illustrated in Table 2. Red show amino acid residues that confer multi-NRTI resistance (all the FDA approved drugs). Green show residues that affect all the FDA approved 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	I210	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; Yahı *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 (Yahı *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

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

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	<u>VAC</u>	<u>DS</u>	M
DRVr	-	-	-	I	F	-	VA	-	V	-	LM	ST	V	-	VAC	-	M
FPVr	-	-	-	<u>I</u>	F	IL	<u>VA</u>	-	V	-	VTALM	ST	V	ATFS	<u>VAC</u>	-	M
INDVr	-	I	-	V	-	IL	V	-	-	L	VTALM	ST	V	<u>AFTS</u>	<u>VAC</u>	S	M
LPVr	-	I	-	I	F	IL	<u>VA</u>	VM	V	-	VTALM	-	V	AFTS	VAC	-	M
NFV	<u>I</u>	<u>I</u>	<u>N</u>	-	F	IL	V	VM	-	L	VTALM	ST	-	AFTS	<u>VAC</u>	<u>DS</u>	<u>M</u>
SQVr	-	I	-	-	-	-	-	VM	-	L	VTALM	ST	-	AT	<u>VAC</u>	S	<u>M</u>
TPVr	-	-	-	I	F	IL	V	-	-	-	VAM	-	-	<u>ATFSL</u>	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

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).

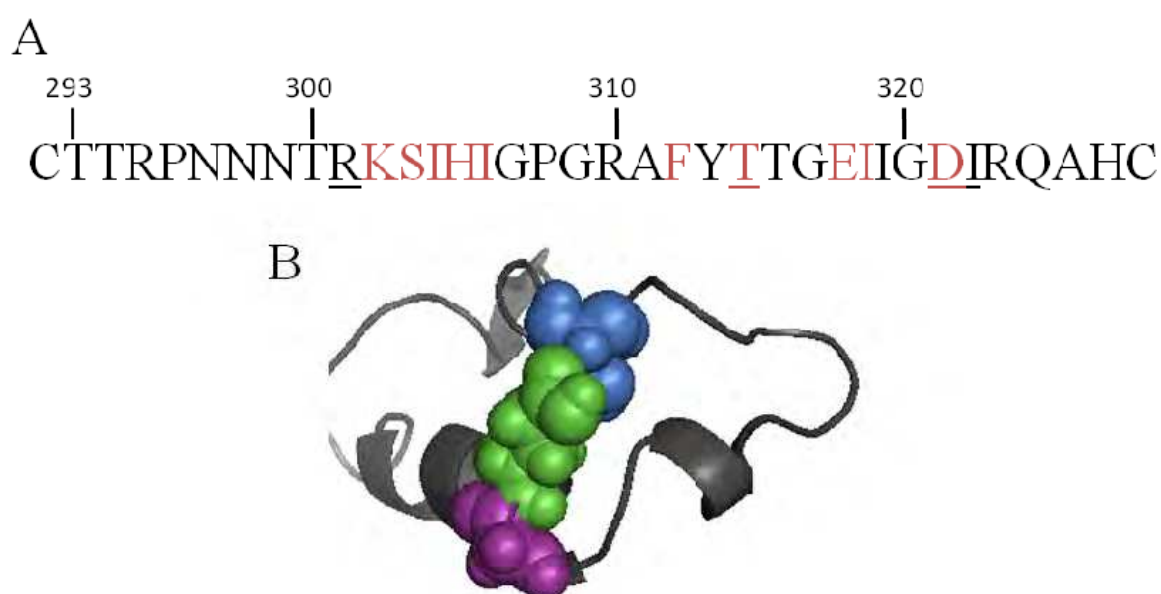


Fig. 7. Illustration of amino acid residues in the V3 loop important for HIV-1 tropism and resistance to maraviroc. (A) The sequence of the V3 variable loop indicating the sites of unique X4 substitution sites when compared to R5 clones (underlined). The red residues indicate the positions where maraviroc 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,

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*

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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