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The Genetic Basis of HIV-1 Resistance to Reverse Transcriptase and Protease Inhibitors

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Abstract

HIV-1 drug resistance is caused by mutations in the reverse transcriptase (RT) and protease enzymes, the molecular targets of antiretroviral therapy. At the beginning of the year 2000, two expert panels recommended that HIV-1 RT and protease susceptibility testing be used to help select antiretroviral drugs for HIV-1-infected patients. Genotypic assays have been developed to detect HIV-1 mutations known to confer antiretroviral drug resistance. Genotypic assays using dideoxynucleoside sequencing provide extensive insight into the presence of drug-resistant variants in the population of viruses within an individual. However, the interpretation of these assays in clinical settings is formidable because of the large numbers of drug resistance mutations and because these mutations interact with one another and emerge in complex patterns. In addition, cross-resistance between antiretroviral drugs is greater than that anticipated from initial *in vitro* studies. This review summarises the published data linking HIV-1 RT and protease mutations to *in vitro* and clinical resistance to the currently available nucleoside RT inhibitors, non-nucleoside RT inhibitors, and protease inhibitors.

Key words

HIV-1. Reverse transcriptase. Protease. Antiretroviral therapy. Drug resistance

The evolution and clinical significance of drug resistance

Fifteen antiretroviral drugs have been approved for the treatment of HIV-1 infection, including six nucleoside RT inhibitors (NRTI), six protease inhibitors (PI), and three non-nucleoside RT inhibitors (NNRTI). In previously untreated individuals with drug-susceptible HIV-1 strains, combinations of three or more drugs from two drug classes can lead to prolonged virus suppression and immunological reconstitution. However, the margin of success for achieving and maintaining virus suppression is narrow. Extraordinary patient effort is required to adhere to drug regimens that are expensive, inconvenient, and often associated with dose-limiting side effects. In addition to these hurdles, the development of drug resistance looms as both a cause and consequence of incomplete virus suppression that threatens the success of future treatment regimens.

The evolution of HIV-1 drug resistance within an individual depends on the generation of genetic variation and on the selection of drug-resistant variants during drug therapy. HIV-1 genetic variability is caused by the inability of HIV-1 RT to proofread nucleotide sequences during replication[1]. It is exacerbated by the high rate of HIV-1 replication *in vivo*, the accumulation of proviral variants during the course of HIV-1 infection, and genetic recombination when viruses with different sequences infect

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Table 1. *Antiretroviral drug resistance in individuals infected with HIV-1 within the preceding 12 months*

Study	Years	Country (Cities)	No.	Resistance test	Percent Resistance*			
					NRTI	NNRTI	PI	MDR
Balotta[9]	1994-1997	Italy (Milan)	38	Genotype	21	3	0	0
Boden[6]	1995-1999	USA (Los Angeles, New York)	80	Genotype with confirmatory phenotype on selected isolates	13	8	3	4
Grant[8]	1996-1999	USA (San Francisco)	118	Genotype with confirmatory phenotype on selected isolates	11	4	4	NA
Harzic[7]	1996-1998	France	158	Genotype	6	1	2	NA
Little[5]	1989-1998	USA (Boston, Dallas, Denver, Los Angeles, San Diego)	141	Phenotype with confirmatory genotype on selected isolates	≥ 3	≥ 1	≥ 1	≥ 1
Salomon[10]	1997-1999	Canada (Montreal)	81	Genotype with confirmatory phenotype on selected isolates	6	4	4	5
Tamalet[11]	1995-1998	France (Marseille, Toulouse)	48	Genotype	17	0	2	2
Yerly[4]	1996-1998	Switzerland (Geneva)	82	Genotype with confirmatory phenotype on selected isolates	10	2	4	4

MDR: multidrug resistance, resistance within more than one class of drugs; NA: not available; NNRTI: non-nucleoside RT inhibitor; No.: number of patients; NRTI: nucleoside RT inhibitor; PI: protease inhibitor
*In these studies, the following mutations were detected and considered genotypic evidence of resistance: for the NRTIs - M41L, D67N, T69D, K70R, L74V, M184V, L210W, T215Y; for the NNRTIs - L100I, K101E, K103N, Y181C, G190A; for the PIs - D30N, M46I, G48V, I54V, V82A, I84V, L90M. In the study by Little *et al.* not all isolates had genotypic testing and the percent resistance represents a lower limit to the prevalence of genotypic resistance in that study.

Table 2. *Correlations between HIV-1 drug-resistance mutations and response to a new antiretroviral treatment (ART)*

Reference	Previous ART	Follow-up ART	Weeks	Effect of baseline mutations on response to the follow-up ART
Holodniy[13]	AZT	AZT + ddI	30	The presence of AZT resistance mutations, particularly T215Y predicted a poor outcome in patients receiving salvage therapy with AZT + ddI, AZT + ddI + NVP, d4T + ddI and maintenance therapy with AZT + 3TC
Mayers[18]	AZT	AZT + ddI or AZT + ddI +NVP	24	
Havlir[14]	IDV + 3TC + AZT	IDV or AZT+3TC	24	
Izopet[16]	AZT + ddC	d4T + ddI	24	
Japour[12]	AZT	AZT or ddI	52	
Kuritzkes[24]	AZT	AZT + 3TC + RTV	48	
Lanier[20]	NRTI, NNRTI, PI	Addition of ABC	12-24	The presence of ≥ 3 AZT resistance mutations, particularly when present with M184V was associated with a poor virological response. The presence of M184V alone was not
Harrigan[21]	≥ 1 PI	RTV + SQV	66	In the papers by Harrigan and Zolopa, the number of mutations at codons 46, 48, 54, 82, 84, and 90 correlated with a worse response to RTV+SQV. In the paper by Zolopa, no virological suppression occurred in patients with ≥ 3 of the mutations. The presence of D30N did not affect response to RTV+SQV
Tebas[22]	NFV	RTV + SQV	24	
Zolopa[19]	≥ 1 PI	RTV + SQV	26	
Para[27]	SQV	IDV	8	Mutations at codons 10, 20, 48, 82, 84, 90 predicted a poor response to indinavir salvage therapy
Condra[26]	NFV	IDV	24	L90M predicted a higher risk of virological failure than D30N
Lawrence[17]	SQV	NFV	16	L90M predicted virological failure with NFV
Klein[28]	NRTI, NNRTI, PI	APV	12	I84V and L90M predicted virological failure; D30N did not
Shulman[25]	NRTI, NNRTI, PI	EFV	24	Among patients with prior NNRTI therapy, those with K103N had no virological response to EFV, those with Y181C had transient responses
Falloon[29]	NRTI, NNRTI, PI	APV + ABC	16	9 heavily treated patients harbouring PI mutations at codons 82 and 90 together with mutations at codons 46 and/or 54 had no virological response to salvage therapy with an APV-containing regimen

ABC: abacavir, ADV: adefovir, APV: amprenavir, AZT: zidovudine, ddI: didanosine, d4T: stavudine, EFV: efavirenz, IDV: indinavir, NFV: nelfinavir, NNRTI: non-nucleoside RT inhibitors, NRTI nucleoside RT inhibitors, NVP: nevirapine, PI: protease inhibitor, RTV: ritonavir, SQV: saquinavir, 3TC: lamivudine.

Table 3. *Prospective intervention studies comparing HIV-1 resistance testing vs. physician guided therapy (PGT)*

Study	Previous Treatment	No.	Weeks	RNA change (log copies/mL)			Comment
				PGT	Genotype	Phenotype*	
GART[32]	≥ 16w of 2 NRTIs and 1 PIs	153	12	–0.61	1.19	ND	
HAVANA[36]	≥ 24w of heavy treatment	274	12	–1.22	–1.45	ND	Expert advice added benefit to genotypic testing (data not shown)
VIRA 3001[33]	≥ 2 NRTIs and 1 PI	237	16	–0.87	ND	–1.23	
VIRADAPT[31]	≥ 24w of NRTI and 12w of PIs	108	24	–0.67	–1.15	ND	Benefit maintained for 9-12 months
Kaiser[34]	> 12w of heavy treatment, NNRTI *naïve*	115	16	No significant differences found between PGT and phenotypic testing			
NARVAL[35]	Heavy treatment (median-7 drugs)	541	12	No significant differences found between PGT, genotypic testing, and phenotypic testing			At 24w, patients receiving genotype had significant benefit

ND: not done, No.: number of patients, NNRTI: non-nucleoside RT inhibitor, NRTI: nucleoside RT inhibitor, PI: protease inhibitor, w: weeks.
* The Virco Antivirogram[42] was used in the VIRA 3001 and Kaiser studies. A different recombinant virus assay was used in NARVAL.

the same cell. As a result, innumerable genetically distinct variants (quasispecies) evolve in individuals in the months following primary infection[2].

The risk of developing drug resistance depends on the size and heterogeneity of the HIV-1 population within an individual, the extent to which virus replication continues during drug therapy, the ease of acquisition of a particular mutation (or set of mutations), and the effect of drug-resistance mutations on changes in drug susceptibility and virus fitness. Some mutations selected during drug therapy confer measurable phenotypic resistance by themselves, whereas other mutations arise to compensate for the diminished replicative activity that can be associated with drug resistance, or produce measurable resistance only when present in combination. Resistant virus strains can also be transmitted between individuals. In the United States and Europe about 10% of new infections are with HIV-1 strains harbouring resistance to at least one of three drug classes[3-11] (Table 1).

An increasing number of studies are showing that the presence of drug resistance before starting a new drug regimen is an independent predictor of virological response to that regimen (Table 2)[12-22 ,23-30]. In addition, prospective controlled studies have shown that patients whose physicians have access to drug resistance data, particularly genotypic resistance data, respond better to therapy than control patients whose physicians do not have access to these assays (Table 3)[31-36]. The accumulation of such retrospective and prospective data has led two expert panels to recommend the use of resistance testing in the treatment of HIV-infected patients[37,38].

HIV drug resistance testing

Current methods for HIV resistance testing include phenotypic drug-susceptibility assays to measure drug inhibition of HIV-1 *in vitro* and genotypic assays that detect mutations known to confer drug resistance. Both tests are generally performed using plasma, because the population of virus within plasma contains the viral variants most recently selected by antiretroviral drug therapy[39-41]. Genotypic susceptibility testing is usually performed by dideoxynucleoside cycle sequencing, whereas phenotypic susceptibility testing is currently performed by two companies that have developed standardised recombinant virus assays amenable to high-throughput performance (Virco, Mechelen, Belgium and ViroLogic, South San Francisco, CA, USA)[42,43].

In research settings, both genotypic and phenotypic tests are required to identify the mechanisms of resistance to new drugs and drug combinations[44]. In clinical settings, both tests, though useful, have limitations. First, the inability to detect minor drug-resistant HIV-1 populations is a recognised limitation of resistance testing using either genotypic or phenotypic methods[38,45,46]. Second, there are gaps in what is known about the clinical significance of certain combinations of mutations

Table 4. *Sources of data on HIV drug resistance mutations*

Genotype-phenotype correlations on laboratory HIV-1 isolates
The pre-clinical evaluation of a new drug often involves culturing a wild-type laboratory HIV-1 isolate in the presence of increasing drug concentrations, and identifying mutations that allow the virus to continue to replicate. Site-directed mutagenesis experiments are done to confirm that the mutations arising during virus passage in the presence of the drug confer drug resistance when introduced into a wild-type virus. Drug-resistance mutations identified by this process acquire widespread acceptance as the predominant mutations responsible for resistance to the drug under evaluation, and are referred to as "canonical" resistance mutations.
Genotype-phenotype correlations on clinical HIV-1 isolates
Laboratory isolates often contain only one or two drug-resistance mutations and rarely reflect the more complicated patterns of mutations observed in clinical isolates from patients receiving combination drug therapy. The complexity of sequences obtained on clinical isolates often precludes site-directed mutagenesis. Instead, statistical associations between drug resistance mutations and *in vitro* resistance are required to elucidate the role of specific mutations or mutation patterns in causing drug resistance.
Genotype-treatment history correlations
Sequences of HIV-1 isolates from patients failing antiretroviral therapy are crucial observations of HIV evolution that show which virus mutations are most significant *in vivo*. Such data are also essential for elucidating the genetic mechanisms of resistance to drugs that are difficult to test *in vitro*.
Genotype-clinical outcome correlations
Correlations between genotype and clinical response to a new regimen constitute the most important type of data needed for physicians attempting to use genotypic data to select the most effective antiretroviral therapy for their patients. However, such data are generally not available and the previous three correlations must be used instead.

and of certain levels of phenotypic drug resistance. Because of the first limitation, clinicians must consider a patient's treatment history when interpreting the results of resistance testing, particularly, in patients with complicated antiretroviral treatment histories, or in patients who have discontinued one or more antiretroviral drugs[47,48]. Because of the second limitation, clinicians must exercise an extraordinary amount of clinical judgment when gauging which drug combinations would be most effective in treating patients that harbour partially resistant HIV-1 strains.

Genotypic tests are used more commonly in clinical settings because of their wider availability, lower cost, and more rapid turnaround. However, genotypic and phenotypic tests often provide complementary information. Phenotypic testing provides a snapshot of the average drug susceptibility of the virus variants within an individual at one time and culminates in a result that is easy to understand. In contrast, genotypic data obtained by sequencing provides better insight into the population of drug-resistant variants within a patient, though such data are complex and often reported in a confusing manner.

Both the richness and complexity of sequence data are revealed by the following examples. Sequences may reveal a mutation as part of a virus mixture, even if the mutation is present in insufficient amounts to affect drug susceptibility in a phenotypic assay. Sequences often reveal mutations that are masked in phenotypic assays. For example, it is common for a mutation that confers resistance to one drug to suppress resistance to a second drug by a different mutation. If most of the circulating virus variants within an individual contain two such antagonistic mutations, a phenotypic assay will report susceptibility to the second drug. In contrast, a genotypic assay will alert the clinician to latent resistance to the second drug that can rapidly evolve into clinical resistance through minimal additional virus selection (e.g. loss of the first mutation). Finally, sequences reveal transitional mutations that do not cause drug resistance by themselves, but which indicate the presence of selective drug pressure and the likelihood that the drug-resistant forms are probably present.

HIV-1 genotypic interpretation

Four types of data must be considered when using HIV-1 RT and protease sequence data to guide therapy (Table 4): (i) Genotypic-phenotypic correlations in laboratory HIV-1 isolates identify the «canonical»drug resistance mutations using site-directed mutagenesis; (ii) Genotype-phenotype correlations in clinical HIV-1 isolates show the *in vitro* phenotypic effect of mutations in the patterns with which they arise *in vivo*; (iii) Genotype-treatment history correlations show the mutations the virus develops to escape from antiretroviral drug pressure *in vivo*; and (iv) Genotype-clinical outcome correlations confirm the clinical significance of mutations by showing how they affect the virological response to a subsequent treatment regimen. Although genotype-clinical outcome correlations are the most relevant considerations for clinicians, they are generally available in only a limited number of situations, making it necessary to also consider the first three types of data.

HIV-1 drug resistance is rarely an all-or-none phenomenon and clinicians usually need the ans-

Table 5. *One possible method for classifying HIV-1 drug susceptibility using genotypic data*

Susceptible	Isolates of this type have not shown reduced drug susceptibility
Potential low-level resistance	Isolates of this type have mutations which do not cause reduced susceptibility by themselves, but may indicate the possibility of previous drug selection
Low-level resistance	Isolates of this type have reduced *in vitro* susceptibility to the drug and/or patients with viruses of this genotype may have a sub-optimal virological response to treatment
Intermediate resistance	The genotype suggests a degree of drug resistance greater than low-level resistance but lower than high-level resistance
High-level resistance	The genotype is similar to that of isolates with the highest levels of *in vitro* resistance and/or patients infected with isolates having similar genotypes generally have little or no virological response to treatment with the drug

Classification used by the Stanford University Hospital Diagnostic Virology Laboratory (Stanford, CA, USA) beginning November 2000.

wer to one of the following two questions: (i) Does the genotype suggest that the patient will respond to a drug in a manner comparable to a patient with a wild-type isolate? (ii) Does the genotype suggest that the patient will obtain any antiviral benefit from the drug? To provide the answers to these two questions, the Stanford Hospital Diagnostic Virology Laboratory reports the following gradations of inferred drug resistance: susceptible, potential low-level resistance, low-level resistance, intermediate resistance, high-level resistance (Table 5). Genotypic interpretations do not necessarily correlate with the inferred level of phenotypic resistance, because the genotypic interpretation is based on clinical data as well as phenotypic data.

The HIV RT and Protease Sequence Database at Stanford University is an online database that contains published data on the first three types of data in table 4 (e.g. genotype-phenotype correlations on laboratory and clinical isolates and genotype-treatment history correlations). Efforts are underway to formally represent summary data from studies containing correlations between genotypes and clinical outcomes[49]. As of November 2000, the database contained 16,300 RT and protease sequences from 2,412 individuals and > 4,000 drug susceptibility results obtained on > 1,000 HIV isolates. Users can retrieve sequence sets matching criteria such as previous drug treatment or presence of specific mutations. Users can also retrieve drug susceptibility results on isolates containing specific mutations.

The HIV RT and Protease Sequence Database also contains two sequence analysis programmes. HIV-SEQ accepts user-submitted RT and protease sequences, compares them to a reference sequence, and uses the differences (mutations) as query parameters for interrogating the sequence database[50]. HIV-SEQ allows users to discover associations between a submitted sequence and previously published sequences containing the same mutations. The database also has a beta test version of a drug-resistance interpretation programme, which accepts user-submitted RT and protease sequences and returns inferred levels of resistance to the 15 available anti-HIV drugs. Drug resistance is inferred using a comprehensive set of rules based on the four types of data in table 4 and hyper-linked to the output of specific database queries.

The remainder of this review summarises the evidence linking mutations to *in vitro* and clinical drug resistance based on the four types of data in table 4. The mutations will be reviewed according to drug class, rather than according to drug, because, although there are exceptions, cross-resistance is so extensive that it is probably more accurate to consider resistance mutations as class-specific rather than drug-specific. Unfortunately, there are many gaps in the published data on HIV-1 drug-resistance mutations. In some cases, the relevant studies have not been performed. In other cases, the studies have been performed but the data are proprietary.

Nucleoside RT inhibitor (NRTI) resistance mutations

The NRTIs are prodrugs that are triphosphorylated by host cellular enzymes and, in that form, compete with natural deoxynucleoside triphosphates (dNTPs) for incorporation into newly synthesised viral DNA chains where they cause chain termination. There are two biochemical mechanisms of NRTI drug resistance. The first mechanism is mediated by mutations that allow the RT enzyme to discriminate against NRTI, thereby preventing their addition to the primer DNA chain (Fig. 1). The second mechanism is mediated by mutations that increase the rate of hydrolytic removal of the chain terminating NRTI and enable continued DNA synthesis[51-54].

In most drug susceptibility assays, the range in susceptibility between wild-type and the most highly drug-resistant viruses (dynamic susceptibility range) is >100-fold for zidovudine and lamivudine and 15 to 20 fold for didanosine, stavudine, zalcitabine, and

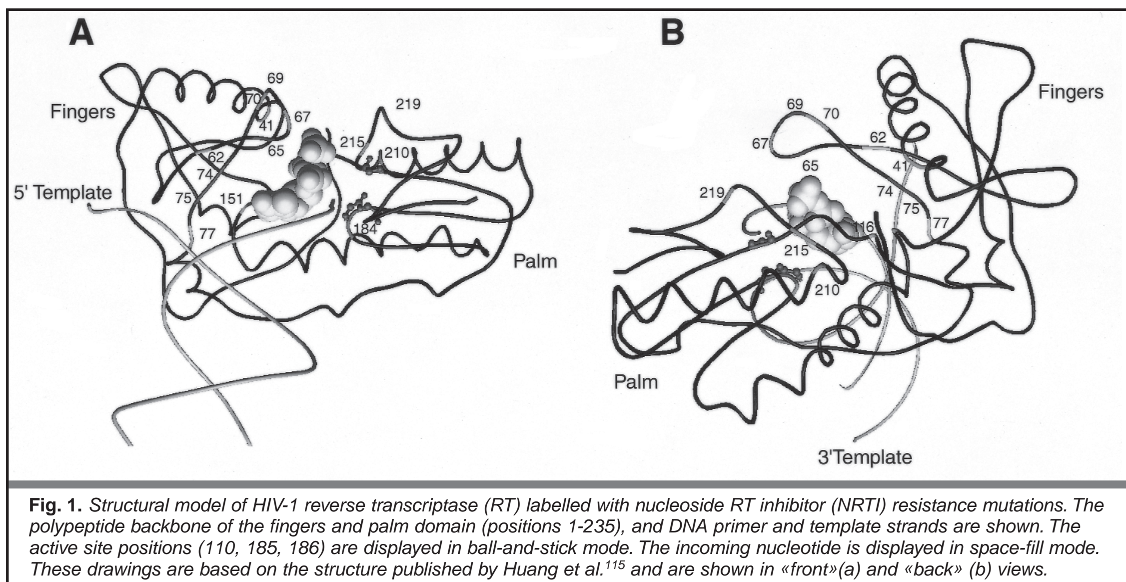

Fig. 1. *Structural model of HIV-1 reverse transcriptase (RT) labelled with nucleoside RT inhibitor (NRTI) resistance mutations. The polypeptide backbone of the fingers and palm domain (positions 1-235), and DNA primer and template strands are shown. The active site positions (110, 185, 186) are displayed in ball-and-stick mode. The incoming nucleotide is displayed in space-fill mode. These drawings are based on the structure published by Huang et al.[115] and are shown in «front»(a) and «back» (b) views.*

abacavir[55]. Mutant isolates from patients failing therapy with zidovudine and lamivudine usually have measurable phenotypic drug resistance. In contrast, mutant isolates from patients failing therapy with stavudine or didanosine are often found to be drug susceptible in phenotypic assays. Fewer published phenotypic data on clinical isolates are available for zalcitabine and abacavir. The difficulty in detecting didanosine resistance is thought to be an artefact of susceptibility testing caused by the inefficient conversion of didanosine to the active compound ddA-triphosphate when stimulated lymphocytes are used for susceptibility testing[56]. Preliminary data suggest that the difficulty in detecting stavudine resistance may also be an artefact of the current susceptibility tests that rely on stimulated lymphocytes containing high dNTP levels[57].

Classical zidovudine resistance mutations

The most common mutations occurring in clinical HIV-1 samples obtained from patients receiving NRTIs were originally identified for their role in causing zidovudine resistance. Various combinations of these mutations which occur at codons 41, 67, 70, 210 ,215, and 219[58-61] have been shown to mediate both ATP and pyrophosphate (PP)-dependent hydrolytic removal (pyrophosphorolysis) of zidovudine monophosphate from a terminated cDNA chain[51-53,62] and cause a compensatory increase in RT processivity[53,63,64]. During the past several years, numerous studies have suggested that these mutations confer resistance not only to zidovudine but also to other nucleoside RT inhibitors, including stavudine, didanosine, and abacavir.

K70R causes low-level (4 to 8-fold) zidovudine resistance and is usually the first drug-resistance mutation to develop in patients receiving zidovudine monotherapy[65,66]. T215Y/F result from a two-base-pair mutation and cause intermediate (10 to 20-fold) zidovudine resistance, and arise in patients receiving dual NRTI therapy or in patients receiving prolonged zidovudine monotherapy[67-69]. T215S/C/D are transitional mutations between wild-type and Y or F that do not cause reduced drug susceptibility but rather indicate the presence of previous selective-drug pressure[70,71]. Mutations at positions 41 and 210 usually occur with mutations at position 215. Mutations at positions 67 and 219 may occur with mutations at position 70 or with mutations at position 215. In patients failing multiple dual nucleoside therapy it is not unusual for isolates to have four, five, or even all six of the classical zidovudine-resistance mutations.

The zidovudine-resistance mutations are not selected during abacavir monotherapy. However, several combinations of these mutations decrease *in vitro* abacavir susceptibility by as much as 5 to 7-fold[72], a level of resistance which should be considered intermediate or high-level, based on the 15 to 20-fold dynamic susceptibility range for this drug. Clinical studies have also shown that virological response to abacavir-containing salvage therapy is inversely related to the number of zidovudine-resistance mutations, particularly in isolates that also have the mutation M184V[29,73,74].

Most of the evidence linking the zidovudine-resistance mutations with stavudine and didanosine resistance is based on clinical data. The most common drug-resistance mutations in patients failing therapy with stavudine or stavudine + didanosine are the classical zidovudine-resistance mutations[16,75-77] and about 10-15% of patients failing therapy with didanosine develop zidovudine-resistance mutations[78,79]. Moreover, previous therapy with zidovudine and the presence of zidovudine-resistance mutations –particularly at position 215– leads to a diminished response to subsequent therapy with either didanosine[12,13,18] or stavudine[16,80] containing regimens.

M184V

M184I/V causes high-level (> 100-fold) lamivudine resistance and emerges rapidly in patients receiving lamivudine monotherapy[81-83]. It is also usually the first mutation to develop in isolates from patients receiving incompletely-suppressive lamivudine-containing regimens[84-88]. M184V is also selected during therapy with abacavir[72,89,90], and less commonly with didanosine[79,91,92] and causes about 2-fold resistance to these drugs[43,79,89,91,93]. M184V alone renders lamivudine ineffective but may not significantly compromise virological response to treatment with abacavir[73,94]. M184V in combination with multiple zidovudine resistance or in combination with mutations at positions 65,74, or 115 leads to *in vitro* and *in vivo* abacavir resistance[72,73,94-96]. The effect of this mutation on subsequent virological response to didanosine-containing regimens has not been studied.

Position 184 is in a conserved part of the RT, close to the active site (two of the catalytic aspartates are at positions 185 and 186). M184V sterically hinders certain NRTI, particularly lamivudine, while still allowing the enzyme to function[97]. The possibility that isolates containing M184V are compromised was suggested by the initial lamivudine monotherapy studies which showed that RNA levels remained about 0.5 log copies below their starting value in patients continuing lamivudine for 6-12 months, despite the presence of lamivudine-resistant isolates containing M184V[98-100]. Several studies have shown that *in vitro* RT enzymes with M184V displayed increased fidelity[101-103] and others have shown that enzymes with M184V display decreased processivity[104-106]. The clinical significance of these biochemical studies is not known and the increased fidelity does not appear to limit the ability of HIV to develop new mutations under continued selective drug pressure[107,108].

M184V reverses T215Y-mediated zidovudine resistance[81,82,109]. For example, HIV-1 isolates containing M41L/T215Y display 64-fold resistance while isolates containing M41L/T215Y and M184V are just 4-fold resistant. This effect is clinically significant and explains the slow evolution of phenotypic zidovudine resistance in patients receiving zidovudine + lamivudine[110,111] but it can be overcome by the presence of four or more zidovudine-resistance mutations[82,95]. This hypersensitivity may be due to the ability of M184V to impair the rescue of chain-terminated DNA synthesis[112] and does not appear to apply to zidovudine resistance caused by Q151M[113]. Whether M184V also reverses the effect of the classical zidovudine mutations on stavudine is not known. However, such a mechanism could also explain the *in vivo* synergy that is observed with the combination of stavudine and lamivudine[114].

Mutations at codons 65, 69, 74, and 75

Positions 64-72 form a loop between the β 2 and β 3 strands in the fingers region of the RT and this loop makes important contacts with the incoming dNTP during polymerisation[115,116]. In addition to the zidovudine-resistance mutations at codons 67 and 70, this region contains several other NRTI resistance mutations. The most common mutations in this region occur at position 69 and include T69D/N/S/A, as well as single and double amino acid insertions. T69D was initially identified as causing resistance to zalcitabine[117], but substitutions at this position have since been reported with each of the available NRTI, and mutations at position 69 contribute resistance to each of the NRTI when they occur with the classical zidovudine-resistance mutations[42,49,118,119]. By themselves, insertions at position 69 cause low-level resistance to each of the NRTI, but isolates containing insertions together with T215Y/F and other zidovudine-resistance mutations have high-level resistance to each of the NRTIs[120-123].

L74V occurs commonly during didanosine and abacavir monotherapy[79,90,124,125] and confers 2 to 5-fold resistance to didanosine and zalcitabine[79,126] and 2 to 3-fold resistance to abacavir[89]. L74V is sufficient to cause virological failure in patients receiving didanosine monotherapy[125] but additional mutations may be required to cause virological failure to abacavir monotherapy. L74V causes hypersensitivity to zidovudine and possibly also to stavudine[126] and is consequently rarely observed in patients receiving dual nucleoside therapy with didanosine/zidovudine or didanosine/stavudine[67,76,77,124,127].

K65R confers intermediate levels of resistance to didanosine, abacavir, zalcitabine and lamivudine[43, 89,90,128-130], but occurs rarely *in vivo*. V75T develops in isolates cultured in the presence of increasing concentrations of stavudine and causes about 5-fold resistance to stavudine and didanosine[131]. V75T occurs rarely, even in patients receiving stavudine. V75I generally occurs in isolates that also have the multi-nucleoside resistance mutation, Q151M. Other mutations at this position include V75M/A.

Multi-nucleoside resistance due to Q151M

Q151M is a two-base-pair change in a conserved RT region that is close to the first nucleotide of the single-stranded nucleotide template[115,132]. This mutation develops in up to 5% of patients who receive dual NRTI therapy with didanosine in combination with zidovudine or stavudine[67,76,77,124,133-135]. Q151M alone causes intermediate levels of resistance to zidovudine, didanosine, zalcitabine, stavudine, and abacavir[113,132,136,137]. Q151M is generally followed by mutations at positions 62, 75, 77 and 116. Isolates with V75I, F77L, F116Y, and Q151M have high-level resistance to each of these NRTI, and low-level resistance to lamivudine.

Other NRTI mutations

An RT polymorphism, G333E, has been reported to facilitate lamivudine resistance in isolates from patients receiving zidovudine and lamivudine that also have multiple zidovudine-resistance mutations[138]. There are few data on the frequency of this mutation

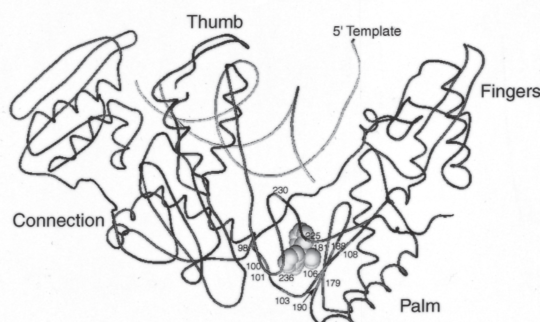

Fig. 2. *Structural model of HIV-1 reverse transcriptase (RT) labelled with non-nucleoside RT inhibitor (NNRTI) resistance mutations. The polypeptide backbone of the complete p66 subunit (positions 1-560), and DNA primer and template strands are shown. This drawing is based on the structure provided by Kohlstaedt et al.[140] in which the RT is co-crystallised with nevirapine, which is displayed in space-fill mode. The positions associated with NNRTI resistance are shown surrounding the hydrophobic pocket to which nevirapine and other NNRTIs bind.*

in treated and untreated persons and there are no data suggesting that this mutation by itself reduces zidovudine susceptibility. E44D and V118I each occur in about 1% of untreated individuals. The prevalence of these two mutations is much higher in isolates obtained from patients receiving dual NRTI combinations, particularly in isolates containing multiple zidovudine-resistance mutations. When present in combination, E44D and V118I cause intermediate lamivudine resistance[139].

Non-nucleoside RT inhibitor (NNRTI) resistance mutations

The NNRTIs bind to a hydrophobic pocket in the RT enzyme close to, but not contiguous with, the active site. These compounds inhibit HIV-1 replication allosterically by displacing the catalytic aspartate residues relative to the polymerase-binding site[140-142]. The mutations responsible for NNRTI resistance are in the hydrophobic pocket to which they bind. A single mutation in this pocket may result in high-level resistance to one or more NNRTIs (Fig. 2). Resistance usually emerges rapidly when NNRTI are administered as monotherapy or in the presence of incomplete virus suppression, suggesting that resistance may be caused by the selection of a pre-existing population of mutant viruses within an individual[143-145]. In most susceptibility assays, the dynamic susceptibility range for each of the three available NNRTIs, nevirapine, delavirdine, and efavirenz is several hundred fold.

HIV-2 and HIV-1 Group O[146-149] are intrinsically resistant to most NNRTIs. In addition, wild-type HIV-1 Group M isolates tend to have greater inter-isolate variability in their susceptibility to NNRTIs than to NRTIs and PIs[150]. Preliminary data suggest that moderate decreases in NNRTI susceptibility (< 10-fold) in the absence of previous NNRTI therapy and known NNRTI-resistance mutations does not interfere with the virological response to an NNRTI-containing HAART regimen[151,152].

NNRTI mutations between codons 98-108

K103N is currently the most clinically important NNRTI resistance mutation because it causes 20 to 50-fold resistance to each of the available NNRTIs[43,153,154]. Although this degree of resistance is less than the highest levels of resistance observed with these drugs, K103N by itself appears sufficient to cause virological failure with each of the NNRTIs[25,155-157]. A different mutation at position 103, K103R, occurs in 2-3% of patients not receiving NNRTIs[49] and has not been reported to cause NNRTI resistance.

V106A causes > 30-fold resistance to nevirapine, intermediate resistance to delavirdine, and low-level resistance to efavirenz[43,153,158-163]. L100I causes intermediate resistance to efavirenz and delavirdine and low-level resistance to nevirapine[43,153,159,160,164,165]. L100I usually occurs with K103N in patients receiving efavirenz and significantly increases efavirenz resistance in these isolates[166]. A98G, K101E and V108I each cause low-level resistance to each of the NNRTI[43,153,154,164].

NNRTI mutations between codons 179-190

Y181C/I cause >30-fold resistance to nevirapine and delavirdine and 2 to 3-fold resistance to efavirenz[43,153,160,164]. Nonetheless, nevirapine-treated patients with isolates containing Y181C generally have only transient virological responses to efavirenz-containing salvage regimens[25,167]. It is not known whether virological failure in this setting is due to low-level Y181C-mediated efavirenz resistance or to the presence of a subpopulation of viruses containing K103N.

Y188C/L/H cause high-level resistance to nevirapine and efavirenz and intermediate resistance to delavirdine[43,153,159,160]. G190A/S cause high-level resistance to nevirapine and efavirenz but do not cause *in vitro* resistance to delavirdine[43,158,159]. There are no clinical data, however, on the usefulness of delavirdine in patients harbouring isolates with these

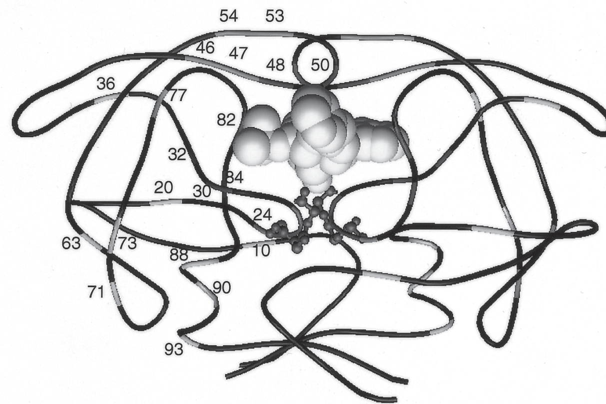

Fig. 3. *Structural model of HIV-1 protease homodimer labelled with protease inhibitor resistance mutations. The polypeptide backbone of both protease subunits (positions 1-99) is shown. The active site (positions 25-27) is displayed in ball-and-stick mode. The protease was co-crystallised with a protease inhibitor, which is displayed in space-fill mode.*

mutations. V179D causes low-level (about 2-fold) resistance to each of the NNRTIs[153,160,165,168].

NNRTI mutations between codons 225-236

P225H causes low-level resistance to efavirenz and possibly nevirapine. By itself, P225H causes delavirdine hyper-susceptibility. However, it usually occurs with K103N in patients receiving efavirenz[154,166,169]. M230L is a recently identified, rare mutation that causes about 20-fold resistance to efavirenz, 40-fold resistance to nevirapine, and 60-fold resistance to delavirdine[170]. P236L is a rare mutation that causes high-level resistance to delavirdine and hyper-susceptibility to nevirapine[154,155,171].

Other mutations

Mutations at codon 138 (e.g. E138K), which have been shown to confer resistance to an experimental group of NNRTIs, the TSAO inhibitors[172], do not cause resistance to the currently approved NNRTI[173].

NNRTI mutation interactions

Mutational interactions within the NNRTI class (e.g. hyper-susceptibility caused by P225H and P236L) have had no clinical implications and there has been no demonstrated benefit of using NNRTIs either in combination or in sequence. Mutational interactions between NNRTI-resistance mutations and NRTI-resistance mutations, however, will probably prove to be clinically relevant. It has been known for several years that Y181C and L100I hyper-sensitise HIV-1 to zidovudine[174,175] and recently it has been shown that some NRTI-resistance mutations appear to hyper-sensitise HIV-1 to certain NNRTIs[176-178]. Although multidrug-resistance to both NRTIs and NNRTIs occurs commonly[161,163,177], these interactions suggest that the number of ways in which HIV-1 can develop simultaneous high-level resistance to both NRTIs and NNRTIs may be restricted. These interactions may also help explain the success of dual NRTI / NNRTI-containing regimens in certain salvage therapy situations[178-180].

Protease inhibitor (PI) resistance mutations

HIV-1 protease is an aspartic protease composed of two non-covalently associated, structurally identical monomers, 99 amino acids in length (Fig. 3).The protease has a substrate-binding cleft that recognises and cleaves 9 different sequences on viral precursor polyproteins. The top of the cleft is covered with a mobile flap that forms a turn over the cleft, but can move away to let substrates enter and products leave. Drug resistance is mediated by structural changes in the substrate cleft that result in a reduction in drug-binding affinity to the mutant target molecule[181-185]. The effects of non-active site mutations are less obvious and appear to involve other mechanisms such as alterations in enzyme catalysis, effects on dimer stability, alterations in inhibitor binding kinetics, or active site re-shaping through long-range structural perturbations[186,187].

Sequence analysis of drug-resistance clones has shown mutations not only within the protease but also at several of the protease cleavage sites[188-190]. Growth-kinetic studies have shown that the cleavage site mutations in some circumstances improve the kinetics of protease enzymes containing drug-resistance mutations and that these mutations appear to be compensatory rather than primary. There have been no reports that changes at cleavage sites alone can cause protease inhibitor resistance and therefore sequencing of these sites is not important for detecting drug resistance in clinical settings.

Mutations at more than 20 positions have been associated with PI resistance, including mutations in the substrate cleft, the flap, other conserved sites of the enzyme, and polymorphic sites. The spectrum of mu-

tations developing during therapy with indinavir, nelfinavir, saquinavir, and ritonavir have been well characterised[186,191-197] but fewer data are available for amprenavir[85,198] and no data are available for lopinavir. The dynamic susceptibility range for indinavir, ritonavir, saquinavir, and nelfinavir is about 100-fold in most drug susceptibility assays[42,43,55,199,200]. The dynamic susceptibility range for amprenavir is about 20 to 30-fold.

Protease substrate cleft mutations

V82A/T/F/S occur predominantly in HIV-1 isolates from patients receiving treatment with indinavir and ritonavir[192,193]. V82A also occurs in isolates from patients receiving prolonged therapy with saquinavir following the development of the mutation, G48V[201,202]. By themselves, mutations at codon 82 cause resistance to indinavir, ritonavir, and lopinavir[192,193,203] but not to nelfinavir, saquinavir, or amprenavir. However, when present with other PI mutations, V82A/T/F/S contributes phenotypic and clinical resistance to each of the PIs[29,95,201,203-205]. V82I occurs in about 1% of untreated individuals with subtype B HIV-1 and in 5-10% of untreated individuals with non-B isolates[49]. Preliminary data suggest that this mutation does not confer resistance to indinavir[206] but its effect on other PIs has not been reported.

I84V has been reported in patients receiving indinavir, ritonavir, saquinavir, and amprenavir[192,193,195,198,199,202] and causes phenotypic[96,192,205,207-211] and clinical resistance to each PI[19,27,204]. G48V occurs primarily in patients receiving saquinavir and rarely in patients receiving indinavir. This mutation causes 10-fold resistance to saquinavir and about 3-fold resistance to indinavir, ritonavir, and nelfinavir[42,201,207,212]. Isolates with mutations at codons 48, 54, and 82 have been tested against each of the PIs except lopinavir and found to have high-level resistance to each[95,96].

D30N occurs solely in patients receiving nelfinavir and confers no *in vitro* or clinical cross-resistance to the other PIs[200,201,207,213]. I50V has been reported only in patients receiving amprenavir as their first PI[85,198]. In addition to causing reduced amprenavir susceptibility, it causes low-level ritonavir resistance of uncertain clinical significance[208,209, 214]. V32I occurs in patients receiving indinavir, ritonavir, and amprenavir. It usually occurs only in association with other PI resistance mutations in the substrate cleft or flap. R8K and R8Q are substrate cleft mutation that cause high-level resistance to one of the precursors of ritonavir (A-77003)[181,215], but they have not been reported with the current PIs.

Protease flap mutations

The protease flap region (positions 46-56) extends over the substrate binding cleft and must be flexible to allow entry and exit of the polypeptide substrates and products[216]. In addition to G48V and I50V, which are also in the substrate cleft, mutations at positions 46, 47, 53, and 54 make important contributions to drug resistance. Mutations at position 54 (generally I54V, less commonly I54T/L/M) contribute resistance to each of the six approved PIs and have been commonly reported during therapy with indinavir, ritonavir, amprenavir, and saquinavir[193,194,196,198,217]. Mutations at position 46 contribute resistance to each of the PIs except saquinavir and have been commonly reported during therapy with indinavir, ritonavir, amprenavir, and nelfinavir[193,194,196,198,217]. Mutations at codon 47 have been reported in patients receiving amprenavir, indinavir, and ritonavir, and often occur in conjunction with the nearby substrate cleft mutation, V32I. F53L has been reported rarely in patients receiving PI monotherapy, but it occurs in more than 10% of patients treated with multiple PIs[49].

Protease mutations at other conserved residues

L90M has been reported in isolates from patients treated with saquinavir, nelfinavir, indinavir, and ritonavir. L90M either contributes to, or directly confers, *in vitro* resistance to each of the six approved PIs and plays a role in causing clinical cross-resistance to each of the PIs[17,19,27,29,199,204,205]. Crystal structures with and without the mutant have shown that the Leu90 side chain lies next to Leu24 and Thr26 on either side of the catalytic Asp25[183,185], but the mechanism by which L90M causes PI resistance is not known.

Mutations at codon 73, particularly G73S, have been reported in 10% of patients receiving indinavir and saquinavir monotherapy and occasionally during nelfinavir monotherapy[49,191]. However, this mutation occurs most commonly in patients failing multiple PIs, usually in conjunction with L90M. Mutations at position 88 (N88D and N88S) commonly occur in patients receiving nelfinavir and occasionally in patients receiving indinavir. By itself, a mutation at this position causes low-level nelfinavir resistance. Together with D30N or M46I, a mutation at this position causes high-level nelfinavir resistance[43,214,218]. N88S (but not N88D) has been shown to hyper-sensitise isolates to amprenavir[218] but the clinical significance of this finding is not known. L24I has been reported only in HIV-1 isolates patients receiving indinavir[217] and has not been shown to confer cross-resistance to other PIs, except possibly lopinavir[204,205].

Polymorphic sites contributing to resistance

Amino acid variants at seven polymorphic positions, including codons 10, 20, 36, 63, 71, 77 and 93 also make major contributions to drug resistance. While these mutations do not cause drug resistance by themselves, some of them contribute to drug resistance when present together with other protease mutations, whereas others compensate for the decrease in catalytic efficiency caused by other protease mutations[219-223].

Mutations at codon 10, 20, 36, and 71 occur in up to 5-10% of untreated persons. However, in heavily treated patients harbouring isolates with

multiple mutations in the substrate cleft, flap, or at codon 90, the prevalence of mutations at these positions increases dramatically. Mutations at codon 10 and 71 increase to 60-80%, whereas mutations at codons 20 and 36 increase to 30%-40%[49,199]. Codon 63 is the most polymorphic protease position. In untreated persons, about 45% of isolates have 63L (considered the subtype B consensus), about 45% have 63P, and about 10% have other residues at this position. However, the prevalence of amino acids other than L increases to 90% in heavily treated patients[49,224]. Mutations at codons 77 and 93 double in prevalence from 15-20% in untreated persons to 30-40% in heavily treated persons[49].

In some HIV-1 subtypes, mutations at codons 10, 20, 36 and 93 occur at higher rates than they do in subtype B isolates[225-227]. It has been hypothesised that individuals harbouring isolates containing multiple-accessory mutations may be at a greater risk of virological failure during PI therapy, however, studies to date have been contradictory[228-230].

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