

# HIV-1 Antiretroviral Resistance

## Scientific Principles and Clinical Applications

*Michele W. Tang and Robert W. Shafer*

Stanford University, Division of Infectious Diseases, Stanford, CA, USA

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### Abstract

The efficacy of an antiretroviral (ARV) treatment regimen depends on the activity of the regimen's individual ARV drugs and the number of HIV-1 mutations required for the development of resistance to each ARV – the genetic barrier to resistance. ARV resistance impairs the response to therapy in patients with transmitted resistance, unsuccessful initial ARV therapy and multiple virological failures. Genotypic resistance testing is used to identify transmitted drug resistance, provide insight into the reasons for virological failure in treated patients, and help guide second-line and salvage therapies.

In patients with transmitted drug resistance, the virological response to a regimen selected on the basis of standard genotypic testing approaches the responses observed in patients with wild-type viruses. However, because such patients are at a higher risk of harbouring minority drug-resistant variants,

initial ARV therapy in this population should contain a boosted protease inhibitor (PI) – the drug class with the highest genetic barrier to resistance.

In patients receiving an initial ARV regimen with a high genetic barrier to resistance, the most common reasons for virological failure are nonadherence and, potentially, pharmacokinetic factors or minority transmitted drug-resistant variants. Among patients in whom first-line ARVs have failed, the patterns of drug-resistance mutations and cross-resistance are often predictable. However, the extent of drug resistance correlates with the duration of uncontrolled virological replication. Second-line therapy should include the continued use of a dual nucleoside/nucleotide reverse transcriptase inhibitor (NRTI)-containing backbone, together with a change in the non-NRTI component, most often to an ARV belonging to a new drug class.

The number of available fully active ARVs is often diminished with each successive treatment failure. Therefore, a salvage regimen is likely to be more complicated in that it may require multiple ARVs with partial residual activity and compromised genetic barriers of resistance to attain complete virological suppression. A thorough examination of the patient's ARV history and prior resistance tests should be performed because genotypic and/or phenotypic susceptibility testing is often not sufficient to identify drug-resistant variants that emerged during past therapies and may still pose a threat to a new regimen. Phenotypic testing is also often helpful in this subset of patients. ARVs used for salvage therapy can be placed into the following hierarchy: (i) ARVs belonging to a previously unused drug class; (ii) ARVs belonging to a previously used drug class that maintain significant residual antiviral activity; (iii) NRTI combinations, as these often appear to retain *in vivo* virological activity, even in the presence of reduced *in vitro* NRTI susceptibility; and rarely (iv) ARVs associated with previous virological failure and drug resistance that appear to have possibly regained their activity as a result of viral reversion to wild type. Understanding the basic principles of HIV drug resistance is helpful in guiding individual clinical decisions and the development of ARV treatment guidelines.

## 1. Introduction

The development of antiretroviral (ARV) combinations potent enough to prevent the emergence of HIV-1 drug resistance was central to the development of successful ARV therapy (ART). Nonetheless, the acquisition and transmission of HIV-1 drug resistance loom as continuing obstacles to successful ART. Patients who acquire or are primarily infected with HIV-1 drug-resistant viruses have fewer treatment options and are at increased risk of morbidity and mortality, particularly in developing countries where choices for ART are limited.<sup>[1,2]</sup>

There are 24 ARV drugs in six classes licensed for the treatment of HIV-1: six nucleoside and

one nucleotide reverse transcriptase inhibitors (NRTIs), five non-nucleoside reverse transcriptase inhibitors (NNRTIs), nine protease inhibitors (PIs), one fusion inhibitor, one CC chemokine receptor 5 (CCR5) antagonist and one integrase inhibitor (table I). Due to a recent expansion in the number of ARVs and ARV classes, virological suppression has become achievable in most patients in whom numerous prior ARV regimens have failed. Identifying and understanding HIV-1 drug resistance can therefore help clinicians avoid minimally active ARVs in favour of newer ARVs that are fully or nearly fully active.

Whereas the principles of drug resistance are the same in all populations, approaches to drug-resistance testing and regimen switching may

**Table 1.** List of currently available US FDA-approved antiretroviral drugs

Generic name	Abbreviation	Brand name (US) <sup>a</sup>
<b>Nucleoside reverse transcriptase inhibitors (NRTIs)</b>		
Abacavir	ABC	Ziagen <sup>®</sup>
Didanosine	ddl	Videx <sup>®</sup>
Emtricitabine	FTC	Emtriva <sup>®</sup>
Lamivudine	3TC	Epivir <sup>®</sup>
Stavudine	d4T	Zerit <sup>®</sup>
Tenofovir	TDF	Viread <sup>®</sup>
Zidovudine	AZT, ZDV	Retrovir <sup>®</sup>
<b>Non-nucleoside reverse transcriptase inhibitors (NNRTIs)</b>		
Delavirdine	DLV	Rescriptor <sup>®</sup>
Efavirenz	EFV	Sustiva <sup>®</sup>
Etravirine	ETR	Intelence <sup>®</sup>
Nevirapine	NVP	Viramune <sup>®</sup>
Nevirapine extended release	NVP XR	Viramune <sup>®</sup> XR <sup>™</sup>
Rilpivirine	RPV	Edurant <sup>®</sup>
<b>Protease inhibitors (PIs)</b>		
Atazanavir	ATV	Reyataz <sup>®</sup>
Darunavir	DRV	Prezista <sup>®</sup>
Fosamprenavir	FPV	Lexiva <sup>®</sup>
Indinavir	IDV	Crixivan <sup>®</sup>
Lopinavir/ritonavir	LPV/r	Kaletra <sup>®</sup>
Nelfinavir	NFV	Viracept <sup>®</sup>
Ritonavir	RTV, /r	Norvir <sup>®</sup>
Saquinavir hard gel caps	SQV	Invirase <sup>®</sup>
Tipranavir	TPV	Aptivus <sup>®</sup>
<b>Integrase inhibitors (INIs)</b>		
Raltegravir	RAL	Isentress <sup>®</sup>
<b>CCR5 antagonist</b>		
Maraviroc	MVC	Selzentry <sup>®</sup>
<b>Fusion inhibitor</b>		
Enfuvirtide (T20)	ENF	Fuzeon <sup>®</sup>

a Many ARVs may have multiple brand names, depending upon the company and location in which they are manufactured. Examples include efavirenz, which is also known as Stocrin<sup>®</sup> in Europe; fosamprenavir, which is also known as Telzir<sup>®</sup> in Europe; lopinavir/ritonavir, which is also known as Aluvia<sup>™</sup> in the developing world; and maraviroc, which is also known as Celsenti<sup>®</sup> in Europe.

**ARV** = antiretroviral; **CCR5** = CC chemokine receptor 5; **/r** indicates boosted by ritonavir.

differ between low-, middle- and high-income countries due to the varying availability of diagnostic tests and ARVs. As a result, clinicians in developing countries must often treat challenging

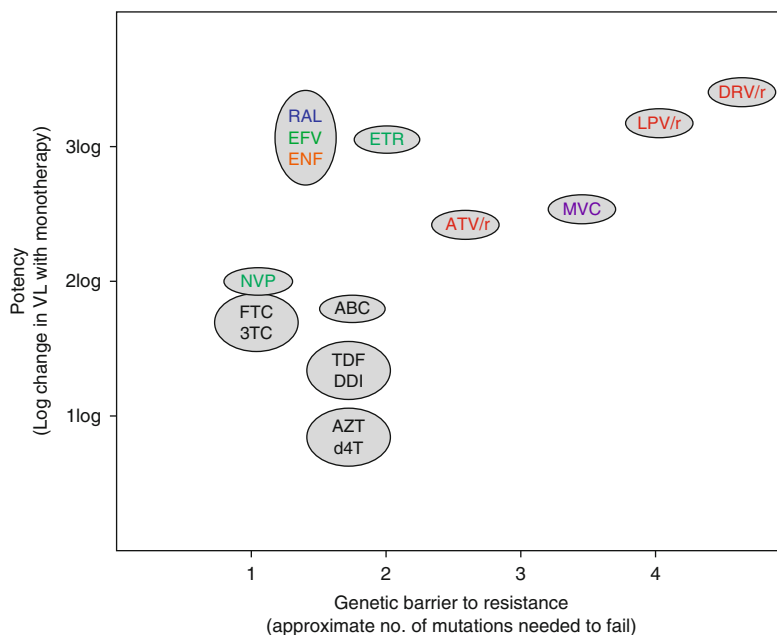
cases of HIV drug resistance with fewer ARV options than those available to their peers in other parts of the world.

In this review, we summarize the efficacy and genetic barriers to resistance associated with different ART regimens, the extent of cross-resistance within each drug class, and approaches to drug-resistance testing. We then show how these principles can be used by clinicians to guide the design of ART regimens for patients with a wide range of treatment histories.

## 2. Biological Basis of Drug Resistance

HIV-1 has a high mutation rate, accumulating nearly one nucleotide mutation per replication cycle.<sup>[3,4]</sup> Although individuals are usually infected with only a single or few original clones,<sup>[5]</sup> an estimated  $10^{10}$  virions are produced each day in untreated individuals, resulting in innumerable virus variants, often called a quasispecies.<sup>[6,7]</sup> The complexity of the HIV-1 quasispecies is also increased by the high recombination rate that occurs whenever more than one viral variant infects the same cell.<sup>[8,9]</sup> In addition, latent virus variants archived in the chromosomes of infected cells may periodically reactivate, further complicating the spectrum of virus variants within infected patients.

The ability to rapidly generate new variants allows HIV-1 to evade the immune system and fosters the development of ARV drug resistance. In fact, the development of antiviral resistance is considered essential to proving that an ARV candidate compound inhibits HIV-1 directly, rather than the host cells in which the virus replicates. HIV-1 drug-resistance mutations occur at the target of therapy and, almost without exception, decrease viral replication fitness. Drug resistance can either be acquired through drug selection pressure (acquired resistance), or transmitted from person to person (transmitted resistance). Naturally occurring drug-resistant viruses (i.e. resistance in viruses that have not been subject to selective drug pressure) are rare. As an important corollary, most drug-resistance mutations are non-polymorphic, meaning that they are rarely observed in untreated patients.



**Fig. 1.** Schematic of genetic barrier to resistance and potency of selected antiretrovirals. The genetic barrier to resistance and potency (antiviral activity) of an antiretroviral determine in large part how susceptible that antiretroviral is to development of HIV-1 resistance. This figure illustrates relative genetic barriers and potencies of commonly used antiretrovirals. Nucleoside/nucleotide reverse transcriptase inhibitors are depicted in black, non-nucleoside reverse transcriptase inhibitors are green, protease inhibitors are red, integrase inhibitors are blue, maraviroc is purple and enfuvirtide is orange.<sup>[13-21]</sup> Refer to table 1 for a full list of drug name abbreviations and definitions. **VL** = viral load.

There is essentially no cross-resistance between drug classes. Even viruses with high levels of resistance to drugs in one ARV class are fully susceptible to drugs belonging to a previously unused ARV class. In the case of the NRTIs and NNRTIs, both of which inhibit reverse transcriptase, there is often *in vitro* synergism, in that NRTI-resistant viruses often increase NNRTI susceptibility<sup>[10,11]</sup> and NNRTI-resistant viruses occasionally increase NRTI susceptibility.<sup>[12]</sup> In contrast, there are often high levels of cross-resistance within each of the drug classes. Most ARV-resistance mutations decrease susceptibility to one or more ARVs of the same class. However, a few drug-resistance mutations have been shown to increase susceptibility to other ARVs of the same class.

For some ARVs, multiple drug-resistance mutations are required to cause decreased susceptibility, while others require just a single mutation. The number of mutations necessary to confer resistance, and the ease or frequency at

which the mutation develops, contributes to the 'genetic barrier to resistance' of the ARV. Drug-resistance mutations can often be categorized as either primary mutations, which directly decrease the susceptibility of the virus to an ARV, or accessory mutations, which enhance viral fitness and further decrease susceptibility. ARVs may differ greatly in their antiviral potency – the extent to which they decrease plasma HIV-1 RNA levels. This intrinsic antiviral potency, together with the genetic barrier to resistance, also influences the vulnerability to resistance of an ARV. Figure 1 illustrates the relative genetic barriers and potencies of representative drugs from each of the six ARV classes.

### 3. Phenotypic and Genotypic Resistance Testing

#### 3.1 Phenotypic Resistance Testing

Phenotypic susceptibility tests measure viral replication in cell culture in the presence of serial

ARV dilutions. Plotting the inhibition of viral replication at increasing ARV concentrations creates a sigmoidal dose-response curve that is usually summarized by the ARV concentration that inhibits viral replication by 50% ( $IC_{50}$ ). The  $IC_{50}$  of an ARV cannot be translated directly into the *in vivo* activity of the ARV because the virus inoculum and cells used in a phenotypic assay often do not reflect *in vivo* conditions. Rather, phenotypic susceptibility testing determines the relative antiviral activity of an ARV against a tested HIV-1 isolate versus against a wild-type control virus. Therefore, drug susceptibility results are reported as levels of fold-resistance, which are calculated by dividing the  $IC_{50}$  of the investigated virus by the  $IC_{50}$  of a control virus. Plasma HIV-1 RNA levels  $\geq 1000$  copies per mL are generally required for phenotypic susceptibility testing.

To determine the significance of a particular reduction in drug susceptibility, assays are characterized by three types of cut-offs for each ARV: (i) the reproducibility of the assay for an ARV ('technical' or 'reproducibility cut-off'); (ii) the range of  $IC_{50}$  values required to inhibit wild-type viruses ('biological cut-off'); and (iii) if sufficient data correlating reductions in drug susceptibility with virological failure are available, then 'clinical cut-offs' can be constructed. For some ARVs, clinical cut-offs may include two values: the fold decrease in susceptibility at which there is some reduction in virological response, and the fold-resistance at which an ARV appears to have minimal, if any, residual antiviral activity.

Two phenotypic assays are available: Phenosense™ (Monogram Biosciences) and Antivirogram™ (Virco). The PhenoSense™ assay is more sensitive than the Antivirogram™ at detecting resistance to ARVs for which small fold differences are clinically significant and is also more reproducible than the Antivirogram™ assay.<sup>[22]</sup>

### 3.2 Genotypic Resistance Testing

Genotypic resistance testing relies on detecting known drug-resistance mutations in the enzymatic targets of antiviral therapy: protease, reverse transcriptase, and, if specially requested, integrase and glycoprotein (gp)41. The standard approach

to genotypic resistance testing is direct polymerase chain reaction (PCR) dideoxynucleotide (Sanger) sequencing. Genotypic testing produces a nucleotide sequence usually encompassing the complete 297 nucleotides (or 99 amino acids) of HIV-1 protease, and the 5' polymerase coding region of HIV-1 reverse transcriptase, usually encompassing amino acid positions 40–240, the part of reverse transcriptase containing the vast majority of NRTI- and NNRTI-resistance mutations. Integrase sequencing is usually ordered as a separate test. The sensitivity of genotypic resistance tests ranges from 100 to 1000 plasma HIV-1 RNA copies per mL, depending upon the assay used.<sup>[23]</sup> At low plasma HIV-1 RNA levels, genotypic resistance testing is likely to be sequencing only a small number of circulating virus variants.

The nucleotide sequence is then translated to its amino acid sequence. The amino acid sequence is then compared either with the sequence of a wild-type subtype B laboratory strain or to a consensus wild-type subtype B amino acid sequence. The differences between a sequenced clinical virus and the reference wild-type sequence generates a list of mutations. Mutations are reported using a shorthand in which each mutation is denoted by the one-letter code for the wild-type reference amino acid, followed by the amino acid position, followed by the one-letter code for the amino acid mutation found in the sequence. For example, the reverse transcriptase mutation 'M184V' indicates that, rather than having a methionine (ATG; M) at position 184, the sequenced virus has a valine (GTG; V). Because direct PCR sequencing is performed on a population of viral genomes, it is not uncommon for the procedure to detect more than one amino acid at a position. For example, the notation 'M184M/V' means that viruses with both ATG (M) and GTG (V) were detected at position 184.

Before the results of a genotypic test can be reported to a physician, the list of mutations in a sequence must be examined to identify those mutations known to be associated with decreased ARV susceptibility. This process is generally performed by the genotypic resistance interpretation system used by the laboratory performing the sequence. There are about ten commonly used

interpretation systems, of which approximately one-half are proprietary and one-half are publicly available online.<sup>[24,25]</sup> There are two commercially available kits for genotypic resistance testing and interpretation: the TRUGENE<sup>®</sup> HIV-1 Genotyping Assay (Siemens, USA)<sup>[26]</sup> and the Celera ViroSeq<sup>®</sup> HIV-1 Genotyping System.<sup>[27]</sup>

### 3.3 Resistance Testing in Clinical Practice

The US Department of Health and Human Services (DHHS),<sup>[28]</sup> International AIDS Society (IAS-USA)<sup>[29]</sup> and European guidelines<sup>[30]</sup> recommend that drug-resistance testing should be performed when a patient is first diagnosed with HIV-1 and in patients with HIV-1 treatment failure. In newly diagnosed patients, a delay in testing would increase the risk that a transmitted drug-resistance mutation would decrease in its proportion relative to the more fit wild-type revertants and no longer be detected by standard genotypic resistance testing, which cannot detect variants present at levels below 20% of the plasma virus population. In patients who defer treatment, repeat genotypic resistance testing should be considered if the patient is at high risk of having been superinfected.<sup>[28]</sup>

Genotypic assays are used more frequently than phenotypic assays because they are less expensive, have a shorter turnaround time and are superior at detecting evolving resistance.<sup>[28]</sup> For example, genotypic tests detect mutations present as mixtures, whereas the impact of such a mixture on a phenotypic test depends on the relative proportions of the wild-type and mutant viruses in the sample, and in some cases decreased susceptibility may not be detected. Likewise, many drug-resistance mutations do not cause drug resistance by themselves but indicate the presence of selective drug pressure and suggest that the viral population within a patient is evolving towards resistance.<sup>[31]</sup> Finally, genotypic tests detect the presence of antagonistic mutations whose effects may be obscured phenotypically. For example, if the tenofovir-resistance mutation K65R and the lamivudine-resistance mutation M184V are present in a virus, a phenotypic test will detect only lamivudine resistance. Although K65R

alone reduces tenofovir susceptibility by about 2.0-fold, the presence of tenofovir resistance in a patient's virus population will be obscured because M184V increases tenofovir susceptibility by about 2.0-fold. A genotypic test alerts the physician to the presence of a major tenofovir-resistance mutation and to the fact that the potential efficacy of using tenofovir in such a patient depends on the continued presence of M184V.

Phenotypic testing is particularly useful to determine the susceptibility of recently approved ARVs for which the genetic correlates of resistance have not yet been well characterized. In clinical practice, it is useful for determining the susceptibility of viruses with complex mutational patterns. This is particularly relevant for selecting a PI for salvage therapy because the clinical significance of many patterns of PI-resistance mutations can be difficult to interpret.

### 3.4 Minority Drug-Resistance Variants

Although genotypic testing is more sensitive than phenotypic testing at detecting mutations present as part of a mixture, standard genotypic testing is unable to reliably detect minority drug-resistant variants present at levels below 20% of the circulating virus population. During the past 10 years, the distribution of minority drug-resistant variants in treatment-naïve and -experienced individuals have been studied using limiting dilution clonal sequencing (also referred to as 'single genome sequencing'),<sup>[32]</sup> point-mutation assays<sup>[33]</sup> and deep sequencing using new massively parallel sequencing technologies.<sup>[34,35]</sup> These studies have shown that minority variant major drug-resistance mutations in the range of 1–20% are frequently detected in treatment-experienced patients and occasionally detected in treatment-naïve patients. The studies that have examined the clinical significance of minority variant drug-resistance mutations have found that minority variant major NNRTI-resistance mutations such as K103N present at levels as low as 1% appear to interfere with the effectiveness of a subsequent NNRTI-containing regimen.<sup>[36,37]</sup>

Methods for detecting minority variants are currently primarily used in research settings be-

cause standard genotypic resistance testing has been used widely in clinical settings for more than 10 years, and a large body of literature supports the reproducibility of this technology and the clinical significance of mutations present at detectable levels. In addition, the two potentially commercially viable approaches to detecting minority variant drug-resistance mutations, point mutation assays and deep sequencing, face technical challenges before they can be used in clinical settings. Finally, the clinical significance of minority populations outside of NNRTI-associated resistance requires further study.

#### 4. Drug Resistance by Antiretroviral (ARV) Classes

##### 4.1 Nucleoside/Nucleotide Reverse Transcriptase Inhibitors (NRTIs)

NRTIs are DNA chain terminators that compete with endogenous deoxy-nucleotide triphosphates (dNTP) for incorporation into a growing viral DNA chain where they cause chain termination. NRTIs are pro-drugs that must be converted to their triphosphate forms by host cellular enzymes.<sup>[38]</sup> The kinetics of NRTI phosphorylation, which varies greatly between different cell types, complicates the *in vitro* assessment of how potent an NRTI will be *in vivo*. The kinetics of NRTI

phosphorylation are also responsible for the highly variable *in vitro* dynamic susceptibility ranges (i.e. the fold-difference between highly drug resistant and wild type). For zidovudine, lamivudine and emtricitabine, highly resistant viruses usually have an IC<sub>50</sub> value more than 100 times higher than wild-type viruses. In contrast, for stavudine, didanosine and tenofovir, highly resistant viruses rarely have an IC<sub>50</sub> value more than five times higher than wild-type viruses.<sup>[39]</sup>

There are two mechanisms of NRTI resistance: (i) discriminatory mutations that enable the reverse transcriptase to discriminate between dideoxy-NRTI chain terminators and the cell's naturally produced dNTPs, thus preventing NRTIs from being incorporated into a growing viral DNA chain; and (ii) primer unblocking mutations that facilitate the phosphorylytic excision of an NRTI-triphosphate that has been added to the growing viral DNA chain. Primer unblocking mutations are also referred to as thymidine analogue mutations (TAMs) because they are selected by the thymidine analogues zidovudine and stavudine.

The most common discriminatory mutations include M184V/I, K65R, K70E/G, L74V, Y115F and the Q151M complex of mutations (figure 2). M184V/I is selected by and causes high-level phenotypic resistance to lamivudine and emtricitabine. It also causes low-level phenotypic cross-resistance to abacavir and, to a lesser extent, didanosine. It

Cons	Discriminatory mutations				Thymidine analogue mutations (TAMs)						MDR mutations	
	184 M	65 K	74 L	115 Y	41 M	67 D	70 K	210 L	215 T	219 K	69 T	151 Q
3TC	<u>VI</u>	R									Ins	M
FTC	<u>VI</u>	R									Ins	M
ABC	<u>VI</u>	<u>R</u>	<u>V</u>	F	L			W	YF		Ins	M
ddI	<u>VI</u>	<u>R</u>	<u>V</u>		L			W	YF		Ins	M
TDF	***	<u>R</u>	*	F	L		R	W	YF		Ins	M
d4T	***	R			L	N	R	W	<u>YF</u>	QE	Ins	M
ZDV	***	***	*		L	N	R	W	<u>YF</u>	QE	Ins	M

**Fig. 2.** Summary of nucleoside/nucleotide reverse transcriptase inhibitor drug-resistance mutations. Mutations are represented by their numeric position and amino acid letter code. The amino acid of the consensus wild-type sequence is represented by letters in the top row, while the amino acids of the mutations are below. Bold and underlining indicates mutations with high-level phenotypic and/or clinical resistance; bold indicates moderate phenotypic and/or clinical resistance; plain text indicates low-level resistance. Asterisks (\*\*\*) represent increased susceptibility to the drug if the mutation is present. Refer to table 1 for a full list of drug name abbreviations and definitions. Adapted from the Stanford University HIV Drug Resistance Database.<sup>[40]</sup> Cons = consensus wild-type; Ins = insertion; MDR = multi-drug resistance.

increases susceptibility to tenofovir and zidovudine. More than any other reverse transcriptase mutation, M184V reduces viral fitness and may be associated with lowering plasma HIV-1 RNA levels 0.5 logs lower than wild-type virus.<sup>[13,41,42]</sup> K65R is the second most important discriminatory mutation. It is selected primarily by tenofovir, and to a lesser extent by stavudine, abacavir and didanosine. K65R causes intermediate resistance to tenofovir, abacavir, didanosine, lamivudine and emtricitabine, low-level resistance to stavudine, and increased susceptibility to zidovudine.

The TAMs include M41L, D67N, K70R, L210W, T215F/Y and K219Q/E. Although they are selected only by zidovudine- and stavudine-containing regimens, the TAMs confer cross-resistance to tenofovir, abacavir and didanosine. The TAMs occur in two distinct but overlapping patterns denoted as type I or type II.<sup>[43]</sup> Type I TAMs include M41L, L210W and T215Y and cause higher levels of phenotypic and clinical resistance to the thymidine analogues and cross-resistance to abacavir, didanosine and tenofovir than do the type II TAMs (D67N, K70R, T215F and K219Q/E). Indeed, the presence of all three type I TAMs markedly reduces the clinical response to abacavir, didanosine and tenofovir.<sup>[44-48]</sup>

The TAMs and discriminatory mutations (with the exception of M184V/I) occur through two separate, opposing pathways.<sup>[49-51]</sup> Viruses that already contain multiple TAMs are more likely to develop additional TAMs when treated with tenofovir, abacavir and didanosine rather than K65R or L74V, the canonical discriminatory mutations associated with these NRTIs. Indeed, the presence of 4–5 TAMs plus M184V is one of the most common patterns of mutations associated with high-level resistance to all of the NRTIs.

Two additional relatively uncommon mutations are also associated with high levels of NRTI cross-resistance: a double amino acid insertion at codon 69 (T69ins), and Q151M. T69ins occurs exclusively in combination with multiple TAMs and in this context causes high-level resistance to all of the NRTIs.<sup>[52-55]</sup> Although the T69ins is only observed in the setting of multiple TAMs, it appears that other NRTIs may also contribute to

its development. Q151M, which usually occurs in combination with several otherwise uncommon accessory mutations (V75I, F77L and F116Y), is associated with high-level resistance to zidovudine, stavudine, abacavir and didanosine, and low-intermediate resistance to tenofovir, lamivudine and emtricitabine.<sup>[39]</sup> Although Q151M does not require any pre-existing mutations, it is a rare mutation that only occurs in prolonged uncontrolled virus replication.

#### 4.2 Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs)

There are four commonly used NNRTIs: nevirapine, efavirenz, etravirine and rilpivirine. Given limited clinical data and complex dosing, delaviridine is rarely used. The NNRTIs inhibit HIV-1 reverse transcriptase allosterically by binding to a hydrophobic pocket close to, but not contiguous with, the reverse transcriptase active site. Nearly all of the NNRTI resistance mutations are within or adjacent to this NNRTI-binding pocket.<sup>[56]</sup> The NNRTIs have a low genetic barrier to resistance. High-level resistance to nevirapine generally requires one mutation, high-level resistance to efavirenz generally requires one to two mutations, and high-level resistance to etravirine requires two mutations.<sup>[57,58]</sup> Preliminary data suggest that the genetic barrier of resistance appears to be lower for rilpivirine than for etravirine, and possibly lower than efavirenz.<sup>[59,60]</sup> There is a high level of cross-resistance within the NNRTI class as a result of two mechanisms: (i) most NNRTI-resistance mutations reduce susceptibility to two or more NNRTIs; and (ii) the low genetic barrier to NNRTI resistance makes it possible for multiple independent NNRTI-resistant lineages to emerge *in vivo*, even if not all of these will be detected by standard genotypic resistance testing.<sup>[61-64]</sup>

The most common NNRTI mutations are L100I, K101EP, K103NS, V106AM, Y181CIV, Y188L, G190ASE and M230L (figure 3). With the exception of L100I, these mutations cause high-level resistance to nevirapine. With the exception of V106A and Y181CIV, these mutations cause intermediate- or high-level resistance to efavirenz. With the exception of K103NS and



<i>Cons</i>	100 L	101 K	103 K	106 V	138 E	181 Y	188 Y	190 G	230 M
<b>NVP</b>	I	<b>EP</b>	<b>NS</b>	<b>AM</b>		<b>CIV</b>	<b>LHC</b>	<b>ASE</b>	<b>L</b>
<b>EFV</b>	I	<b>EP</b>	<b>NS</b>	<b>AM</b>		C	<b>LHC</b>	<b>ASE</b>	<b>L</b>
<b>ETR</b>	I	<b>EP</b>			K	<b>CIV</b>	L	<b>ASE</b>	<b>L</b>
<b>RPV</b>	I	<b>EP</b>			K	<b>CIV</b>	<b>L</b>	<b>ASE</b>	<b>L</b>

**Fig. 3.** Summary of non-nucleoside reverse transcriptase inhibitor drug-resistance mutations. Mutations are represented by their numeric position and amino acid letter code. The amino acid of the consensus wild-type sequence is represented by letters in the top row, while the amino acids of the mutations are below. Bold and underlining indicates high-level phenotypic and clinical resistance (probable contra-indication); bold text indicates moderate phenotypic or clinical resistance; plain text indicates contributes to resistance. Additional mutations: nonpolymorphic, usually accessory: V179F (NVP, ETR), P225H (EFV), F227L (NVP), E138QG (ETR, RPV), K238TN (EFV, NVP), Y318F (NVP), N348I (NVP); nonpolymorphic, rare: K101HN, K103HT, G190QTCV, F227C; polymorphic accessory: V90I, A98G, V108I, E138A, V179DE, H221Y. Genotypic susceptibility scores: ETR (Tibotec): 181IV (3.0); 100I, 101P, 181C, 230L (2.5); 90I, 138A, 179F, 190S (1.5); 98G, 101EH, 179DT, 190A (1.0); <2.5 susceptible; 2.5–3.0 intermediate; >3.0 high-level. Refer to table I for a full list of drug name abbreviations and definitions. Adapted from the Stanford University HIV Drug Resistance Database.<sup>[40]</sup> **Cons** = consensus wild-type.

V106AM, the most common NNRTI mutations are also associated with decreased etravirine and/or rilpivirine susceptibility. However, high-level etravirine resistance generally requires the uncommon 2-bp mutations Y181I/V or two or more NNRTI-resistance mutations.<sup>[58]</sup>

Although etravirine and rilpivirine have similar chemical structures, rilpivirine has a lower genetic barrier to resistance than etravirine and is approved solely for first-line therapy. Indeed, a newly recognized mutation (E138K) has been shown to emerge in about one-half of patients developing virological failure while receiving rilpivirine.<sup>[65]</sup> E138K also appears to confer some cross-resistance to etravirine, efavirenz and nevirapine.<sup>[66,67]</sup> In an unusual example of ‘cross-talk’ between the NRTI- and NNRTI-resistance mutations, patients with tenofovir/emtricitabine/rilpivirine failure are more likely to develop M184I (rather than the more common mutation, M184V) in combination with E138K.<sup>[59,68–70]</sup> Y188L is another mutation selected by rilpivirine. It causes high-level resistance to rilpivirine, nevirapine and efavirenz but only causes potential low-level resistance to etravirine.

Patients receiving efavirenz-containing regimens are less likely than those receiving nevirapine-containing regimens to develop cross-resistance to etravirine because K103N, the most prevalent mutation selected by efavirenz<sup>[71]</sup> does not confer resistance to etravirine. In contrast, Y181C, which commonly occurs with nevirapine but not with efavirenz-containing regimens, confers partial cross-resistance to etravirine.<sup>[72]</sup> Given the

complexity of determining residual activity to etravirine when NNRTI-associated mutations are present, a ‘genotypic susceptibility score’ (GSS) has been developed to predict etravirine susceptibility (figure 3).<sup>[58]</sup>

#### 4.3 Protease Inhibitors (PIs)

The PIs are competitive active site inhibitors of HIV-1 protease that prevent the enzyme from processing the Gag and Gag/Pol polyprotein precursors necessary for viral maturation.<sup>[73]</sup> Although nine PIs have been approved by the US FDA, ritonavir is used solely at sub-therapeutic doses to inhibit cytochrome P450 (CYP)3A, to ‘boost’ the levels of other PIs (denoted by ‘/r’ following the PI). Nelfinavir, which cannot be boosted by ritonavir, is rarely used because of its suboptimal antiviral efficacy. Indinavir/r, although it has potent antiviral activity, is associated with high rates of renal toxicity and is now rarely used or studied. Among the remaining six PIs, three are commonly used: atazanavir/r, lopinavir/r and darunavir/r.<sup>[28,74]</sup> Atazanavir/r and darunavir/r are components of first-line recommended therapy in the current DHHS guidelines, while lopinavir/r is considered an acceptable alternative. Of the remaining PIs, fosamprenavir/r and saquinavir/r are considered acceptable options for first-line therapy but have been infrequently studied in routine clinical settings. Tipranavir/r is not recommended in first-line therapy<sup>[75]</sup> but has a niche role in salvage therapy because it is the only PI that oc-

asionally retains clinically significant activity against darunavir/r-resistant viruses.<sup>[76,77]</sup>

There are many PI-resistance mutations. Major PI-resistance mutations by themselves typically reduce susceptibility to one or more PIs. Accessory PI-resistance mutations compensate for the decreased replication associated with major mutations and further reduce PI susceptibility in combination with one or more of the major PI-resistance mutations. Most PI-resistant viruses also require at least one compensatory Gag cleavage site mutation.<sup>[73]</sup>

The major PI mutations are D30N, V32I, M46IL, G48VM, I50VL, I54VTALM, L76V, V82ATFS, I84V, N88S and L90M (figure 4). D30N, which is selected by nelfinavir, and I50L, which is selected by atazanavir, are notable for causing high-level resistance to just one PI. In contrast, each of the remaining major mutations reduce susceptibility to two or more PIs. PI cross-resistance is complex as a result of the large number of PI-resistance mutations and the fact that different mutations at the same position can have markedly different effects on PI susceptibility.<sup>[78]</sup> This is particularly the case for mutations at positions 50, 54 and 82.

The requirement for one or more major and one or more accessory PI-resistance mutation in combination with Gag cleavage site mutations explains

the high genetic barrier for ritonavir-boosted PIs. Lopinavir/r and darunavir/r have the highest genetic barriers to resistance, with a minimum of three to four mutations required for high-level lopinavir/r resistance and even more mutations required for high-level darunavir/r resistance.<sup>[78,79]</sup> Proof-of-principle studies have shown that, although lopinavir/r alone is not as effective as lopinavir/r plus two NRTIs for initial ARV therapy, it is sufficient to suppress HIV-1 replication to levels below detection for more than 1 year in the majority of ARV-naive patients.<sup>[80]</sup> There are no studies of darunavir/r monotherapy in ARV-naive patients, but the MONET (Monotherapy in Europe with TMC114) and MONOI studies showed high rates of continued virological suppression when virologically suppressed patients were simplified to darunavir/r monotherapy.<sup>[81,82]</sup> Although tipranavir/r is often active against lopinavir/r-resistant viruses and occasionally active against darunavir/r-resistant viruses, its genetic barrier to resistance is poorly understood.<sup>[78,83]</sup> Indeed, much of its activity against highly resistant viruses may result from the fact that many protease mutations that confer resistance to lopinavir/r and darunavir/r increase tipranavir/r susceptibility.

PI-resistance mutations rarely emerge in patients receiving first-line therapy with a boosted PI.<sup>[84,85]</sup> This suggests that virological failure in such patients

<i>Cons</i>	30 D	32 V	46 M	47 I	48 G	50 I	54 I	76 L	82 V	84 I	88 N	90 L
ATV/r		I	IL	V	VM	<u>L</u>	VTAM		ATFS	<u>V</u>	<u>S</u>	M
DRV/r		I		VA		V	LM	V	F	V		
FPV/r		<u>I</u>	IL	<u>VA</u>		<u>V</u>	<u>VTALM</u>	<u>V</u>	ATFS	<u>V</u>		M
IDV/r		I	IL	VA			VTA	V	<u>ATFS</u>	<u>V</u>	S	M
LPV/r		I	IL	<u>VA</u>	VM	V	VTALM	V	ATFS	V		M
NFV	<u>N</u>		<u>IL</u>	VA	<u>VM</u>		VTALM		ATFS	<u>V</u>	<u>DS</u>	<u>M</u>
SQV/r					<u>VM</u>		VTAM		AT	<u>V</u>	S	M
TPV/r		I	IL	VA			VTAM		LT	V		

**Fig. 4.** Summary of protease inhibitor (PI) drug-resistance mutations. Mutations are represented by their numeric position and amino acid letter code. The amino acid of the consensus wild-type sequence is represented by letters in the top row, while the amino acids of the mutations are below. Bold and underlining indicates significant phenotypic or clinical resistance (probable contraindication); bold indicates significant contribution to resistance; plain text indicates primarily accessory contribution to resistance. Common accessory mutations: L101VF, V11I, K20TVI, L23I, L24IF, L33F, K43T, F53L, Q58E, A71VTIL, G73STCA, T74PS, N83D, L89V. Hypersusceptibility mutations: I50L increases susceptibility to all PIs except ATV. I50V and I54L to TPV. L76V to ATV, SQV and TPV. N88S to FPV. Genotypic susceptibility scores: DRV (Tibotec):111, 32I, 33F, 47V, 50V, 54LM, 74P, 76V, 84V, 89V; <3 susceptible; 3–4 low/intermediate; >4 high level. TPV (Boehringer): 47V (+4), 74P (+4), 82LT(+4), 83D (4), 58E (3), 84V (3), 36I (2), 43T (2), 54AMV (2), 10V (1), 33F (1), 46L (1), 24I (–2), 76V (–2), 50L/V (–4), 54L (–6); <4 susceptible; 5–10 intermediate; >10 high level. Refer to table I for a full list of drug name abbreviations and definitions. Adapted from the Stanford University HIV Drug Resistance Database.<sup>[40]</sup> **Cons** = consensus wild-type.

may be in part due to decreased adherence and probably other variables that have yet to be identified. The importance of adherence is supported by anecdotal reports that many of the virological failures lacking PI-resistance mutations have been re-suppressed with their initial PI-containing regimen.

In contrast to the rarity of PI-resistance mutations in patients receiving an initial PI-containing ARV regimen, multiple PI-resistance mutations have developed in the population of heavily treated patients who began ARV therapy in the pre-ART era. These patients typically were treated initially with mono- and dual-nucleoside therapy, followed by sequential un-boosted first-generation PIs. Many of these patients now harbour viruses with high-level resistance to multiple PIs, including a subset with intermediate to high levels of resistance to lopinavir/r, tipranavir/r and darunavir/r.

#### 4.4 Integrase Inhibitors (INIs)

Following reverse transcription, integrase cleaves conserved dinucleotides from the 3' ends of double-stranded HIV-1 DNA, leaving dinucleotide overhangs on both ends of the genome ('the 3'-processing reaction'). Integrase remains bound to each of the 3' ends, circularizing the virus, and translocating it to the nucleus. In the nucleus, integrase catalyzes integration of viral double-stranded DNA into the host chromosome (the 'strand-transfer reaction').<sup>[86]</sup> Although integrase catalyzes both the 3'-processing and strand-transfer reactions, only those compounds that specifically inhibit strand transfer have been effective INIs because INIs bind only to those enzymes that are already bound to viral DNA.<sup>[87]</sup> INIs bind close to the active site of the enzyme and disrupt the correct positioning of the viral DNA relative to the active site as well as the interaction of the enzyme with two essential magnesium ions.

One INI, raltegravir, has been approved by the FDA for use in first-line and salvage antiretroviral therapy. A second INI, elvitegravir, is likely to be approved this year as part of a fixed-dose formulation with tenofovir/emtricitabine and cobicistat, a CYP inhibitor.<sup>[88]</sup> Dolutegravir (formerly S/GSK1349572) is currently being

<i>Cons</i>	66 T	92 E	143 Y	147 S	148 Q	155 N
RAL	A	Q	<u>R</u> CH	G	<u>HR</u> K	<u>H</u>
EVG	<u>I</u> AK	Q		<u>G</u>	<u>HR</u> K	<u>H</u>
DTG		Q			HRK	

**Fig. 5.** Summary of integrase inhibitor drug-resistance mutations. Mutations are represented by their numeric position and amino acid letter code. The amino acid of the consensus wild-type sequence is represented by letters in the top row, while the amino acids of the mutations are below. Bold and underlining indicates >10- to 20-fold decreased susceptibility (probable contraindication); bold text indicates significant contribution to phenotypic and/or clinical resistance; plain text indicates <5- to 10-fold decreased susceptibility. Refer to table 1 for a full list of drug name abbreviations and definitions. Adapted from the Stanford University HIV Drug Resistance Database.<sup>[40]</sup> **Cons** = consensus wild-type.

evaluated in phase III studies for raltegravir-naïve and -experienced patients and will likely be approved in 2013.<sup>[89]</sup>

Raltegravir resistance occurs by three main, occasionally overlapping, mutational pathways: N155H followed by E92Q; Q148HRK+G140SA; and Y143CR+T97A (figure 5). Each of these pairs of mutations are often accompanied by other accessory mutations (reviewed in Blanco et al.<sup>[90]</sup>). Several integrase mutations, including N155H, Q148R and Y143R have been shown in site-directed mutants to reduce raltegravir susceptibility more than 10-fold. Many pairs of mutations decrease raltegravir susceptibility >100-fold. These mutations appear to interfere with the inhibitor binding to the integrase enzyme and the essential magnesium ions.<sup>[91]</sup>

With the exception of Y143CR, most raltegravir-resistance mutations confer cross-resistance to elvitegravir. Likewise, it appears that most elvitegravir-resistance mutations are likely to confer cross-resistance to raltegravir. Dolutegravir appears to have a higher genetic barrier to resistance than raltegravir and elvitegravir. Indeed, preliminary data suggest that only the combination of Q148+G140 mutations reduce dolutegravir susceptibility and that complete dolutegravir cross-resistance may require at least one additional mutation.<sup>[92,93]</sup>

Although most patients developing virological failure while receiving raltegravir or elvitegravir have two or more INI-resistance mutations, the

fact that a single mutation can reduce raltegravir (and elvitegravir) susceptibility more than 10-fold suggests that these inhibitors have a low genetic barrier to resistance.<sup>[94,95]</sup> Further evidence for a low genetic barrier comes from the fact that raltegravir often selects for more than one INI-resistant lineage within a patient. Indeed, although viruses belonging to the N155H pathway often emerge early following virological failure, they are often replaced within weeks by viruses stemming from the Q148 and less commonly the Y143 pathways.<sup>[96,97]</sup> Despite the importance of viral fitness in the evolution of raltegravir-resistant variants, several studies have shown that there is little virological benefit in continuing raltegravir once resistance has developed.<sup>[98,99]</sup>

#### 4.5 CC Chemokine Receptor 5 (CCR5) Antagonists

The small-molecule inhibitor maraviroc allosterically inhibits the binding of HIV-1 gp120 to the host CCR5 (R5) co-receptor. Maraviroc in combination with two NRTIs is recommended as an alternate regimen for first-line therapy by the 2010 IAS-USA<sup>[29]</sup> and 2011 DHHS treatment guidelines.<sup>[30]</sup> It has also been used successfully in salvage regimens with other active ARVs in patients with CCR5-tropic virus.<sup>[100,101]</sup>

The most common reason for maraviroc failure is the presence of undetected minority variant CXCR4 chemokine receptor 4 (CXCR4 or X4) tropic viruses.<sup>[102,103]</sup> Although HIV-1 can also develop maraviroc resistance via mutations that allow HIV-1 gp120 to bind to an inhibitor-bound R5 receptor, reports of such resistance have been documented primarily *in vitro* and in only a small number of clinical viruses.<sup>[104,105]</sup> The paucity of primary CCR5-antagonist resistance has made it impossible to characterize the responsible genetic mechanism of resistance. However, such resistance can be assessed phenotypically.<sup>[106]</sup>

More than 80% of patients are initially infected with HIV-1 viruses that are solely R5 tropic. X4 tropic viruses usually emerge in the later stages of HIV-1 infection. About 50% of patients chronically infected with HIV-1 are eventually found to harbour X4 tropic viruses.<sup>[107-110]</sup> How-

ever, when X4-tropic viruses emerge, they are often present in low proportions relative to R5 tropic viruses.<sup>[108,111]</sup>

A tropism assay should be performed prior to using maraviroc, to confirm that a patient is not infected with X4 tropic variants. Tropism can be detected phenotypically or genotypically. The phenotypic Trofile<sup>®</sup> test (Monogram) assesses the tropism of complete *env* genes amplified from patient samples using reporter cell lines expressing CCR5 or CXCR4.<sup>[112]</sup> Given a sufficiently high plasma virus level, it has the sensitivity to detect X4 variants at populations less than 1%.<sup>[113]</sup>

Genotypic testing for tropism uses algorithms that predict tropism from the envelope V3 loop sequence. Standard genotypic testing using Sanger sequencing has a specificity of ~85% and sensitivity of 50–70%. The low sensitivity of standard genotypic testing arises from the fact that X4 tropic variants are often present at levels below the 20% sensitivity of standard sequencing and because some of the determinants of tropism lie outside of the V3 loop. However, the sensitivity of genotypic testing can be improved by sequencing multiple independent aliquots of viral complementary DNA (cDNA) or by using the Roche/454 Life Sciences deep sequencing technology.<sup>[114]</sup> Indeed, comparative analyses of baseline samples from the MERIT (Maraviroc versus Efavirenz in Treatment-Naive Patients) and MOTIVATE (Maraviroc plus Optimized Background Therapy in Viremic, ART-Experienced Patients) trials suggests that sensitivity and specificity of deep sequencing approaches that of the enhanced-sensitivity Trofile<sup>®</sup> assay.<sup>[115-117]</sup> Several clinical laboratories now offer either standard V3 loop sequencing of multiple cDNA aliquots or Roche/454 Life Sciences deep sequencing to detect minor X4 tropic viruses.

#### 4.6 Fusion Inhibitors

Enfuvirtide, the only approved fusion inhibitor, inhibits the interaction of gp41 hairpin formation, the process by which two complementary parts of gp41 fold onto one another, shortening the protein and bringing the viral and host cell membranes together. The antiviral activity of

enfuvirtide is similar to that of the most active ARVs such as efavirenz, lopinavir/r and raltegravir.<sup>[118]</sup> Despite its high potency and unique mechanism of action, enfuvirtide use is limited because it is administered subcutaneously, and frequently elicits painful injection site reactions. With the approvals of raltegravir and maraviroc, enfuvirtide use has decreased and it has been reserved for the most highly treatment-experienced patients.

Enfuvirtide has a low genetic barrier, and resistance develops rapidly in salvage therapy patients not receiving a sufficient number of additional active drugs.<sup>[119,120]</sup> Mutations in gp41 codons 36–45, the region to which enfuvirtide binds, are primarily responsible for enfuvirtide resistance.<sup>[121–123]</sup> The key enfuvirtide mutations are G36DEV, V38EA, Q40H, N42T and N43D. A single enfuvirtide-resistance mutation usually reduces enfuvirtide susceptibility about 10-fold, whereas two mutations usually reduce susceptibility about 100-fold. Genotypic and phenotypic enfuvirtide-resistance testing is commercially available. Although enfuvirtide-resistant variants have decreased fitness,<sup>[124]</sup> maintaining enfuvirtide in patients who have decreased enfuvirtide susceptibility has provided minimal, if any, virological benefit.<sup>[118]</sup>

## 5. Non-Subtype B Viruses

Antiretroviral drugs are as effective in non-subtype B group M HIV-1 viruses as they are in the subtype-B viruses primarily found in the US and Europe.<sup>[125–127]</sup> Mutations that cause resistance in subtype B viruses also cause resistance in each of the other subtypes. However, subtypes vary in their propensities to develop specific mutations as a result of three potential factors: (i) inter-subtype differences in codon usage; (ii) inter-subtype amino acid differences that result in subtle structural differences in the targets of therapy; and (iii) inter-subtype differences in the sequence context surrounding a nucleotide at which a substitution results in a drug-resistance mutation.

The propensity for subtype C viruses to develop V106M during NNRTI treatment – rather than V106A, which is more commonly observed in subtype B viruses – results from the fact that V106

is encoded by GTA in subtype B viruses and GTG in subtype C viruses. A single G-to-A transition (the most common reverse transcriptase error) at the first position of codon 106 in subtype C viruses results in V106M, which confers high-level efavirenz and nevirapine resistance. In contrast, in subtype B viruses, V106M requires two nucleotide substitutions (GTA → ATG) and therefore occurs uncommonly.<sup>[128,129]</sup> A similar phenomenon has been observed at protease codon 82 in subtype G viruses, which are much more likely than subtype B viruses to develop the poorly characterized mutation V82M.<sup>[130]</sup>

Differences in the amino acids between subtypes can create subtle differences in the structural micro-environment that may predispose HIV-1 to different mutations under similar selective pressure. For example, subtype B-infected patients receiving nelfinavir are more likely to develop D30N than are those with viruses belonging to subtypes C, F, G and CRF01\_AE, which are more likely to develop L90M or N88S.<sup>[131–133]</sup> *In vitro* studies have suggested that inter-subtype differences in the consensus amino acid at position 89 with leucine (L) in subtype B and methionine (M) in most other subtypes are responsible for the different patterns of observed resistance.<sup>[134]</sup>

Several lines of evidence suggest that patients infected with subtype C viruses who are treated with stavudine/lamivudine/efavirenz or stavudine/lamivudine/nevirapine may be more prone than patients infected with subtype B viruses to develop the NRTI-resistance mutation, K65R.<sup>[135,136]</sup> Elegant biochemical research suggests that the unique sequence context in the region of K65R, specifically – a span of five consecutive adenosines preceding the adenosine at the second position in the K65 codon – renders it more likely to be mutated during reverse transcription.<sup>[137,138]</sup>

## 6. Transmitted Drug Resistance

Although drug-resistant mutations usually reduce viral fitness, most transmitted drug-resistant viruses revert to wild type gradually over a period of several years.<sup>[139–141]</sup> This slow reversion occurs because most new infections are monoclonal

and during the expansion and diversification of this clone early in infection, it acquires multiple cytotoxic T-lymphocyte (CTL)-escape mutations, which may provide the drug-resistant virus a temporary selective advantage against wild-type viral revertants.<sup>[5,142]</sup> However, some mutations, such as M184V and T215Y, appear more likely than others to revert to wild type.<sup>[143]</sup> In contrast, the NNRTI-resistance mutations, particularly K103N, appear highly likely to persist for a prolonged period following initial infection. Few data are available for the rates of reversion for most PI- and INI-resistance mutations.

Recent studies show that the prevalence of transmitted drug-resistant virus ranges from 12% to 15% in the US.<sup>[144-146]</sup> In Europe, Canada and other industrialized countries, the risk of transmitted drug resistance is approximately 10%.<sup>[147-151]</sup> In resource-limited regions, where ARV programmes were scaled up in early- to mid-2000, most studies have reported that fewer than 5% of new infections are caused by viruses with evidence of transmitted drug resistance. However, several studies have reported prevalences as high as 10% in localized areas,<sup>[152-155]</sup> and two recent meta-analyses suggest that transmitted NNRTI resistance is gradually increasing in resource-limited settings.<sup>[156,157]</sup> Therefore, in regions where initial genotypic resistance testing is not readily available, surveillance of primary drug resistance is particularly important to identify populations at high risk for transmitted resistance, for whom ART recommendations may need to be modified or for whom baseline resistance testing should be considered.<sup>[158]</sup>

The transmission of drug-resistant HIV-1 strains is associated with a high risk of virological failure.<sup>[159,160]</sup> Although patients infected with a virus containing one or more transmitted drug-resistance mutations are likely to be at higher risk of harbouring additional minority drug-resistant variants, the virological response to a regimen selected based on the results of standard genotypic testing appears to be almost as effective as the initial treatment of a patient without transmitted resistance.<sup>[2,140,161,162]</sup> Nonetheless, patients with transmitted NNRTI resistance should probably be treated with a regimen with a high genetic

barrier such as a boosted PI combined with two NRTIs. Regimens with lower genetic barriers such as raltegravir plus two NRTIs or rilpivirine plus two NRTIs may pose an increased risk of virological failure as a result of additional minority NRTI- and NNRTI-resistant variants.<sup>[61,163]</sup>

## 7. First-Line ARV Therapy Failure

The 2011 DHHS guidelines<sup>[28]</sup> recommend tenofovir/emtricitabine in combination with efavirenz, atazanavir/r, darunavir/r or raltegravir for first-line therapy in ARV-naive patients infected with wild-type viruses. Abacavir/lamivudine was recently downgraded to an alternative NRTI combination, because a recent large clinical trial comparing abacavir/lamivudine with tenofovir/emtricitabine in combination with efavirenz or atazanavir/r showed that those patients with viral loads higher than  $10^5$  copies/mL who received abacavir/lamivudine were at increased risk of virological failure.<sup>[164]</sup> The 2010 World Health Organization (WHO) HIV treatment guidelines<sup>[165]</sup> recommend zidovudine/lamivudine, tenofovir/lamivudine or tenofovir/emtricitabine in combination with nevirapine or efavirenz as first-line therapy. Stavudine/lamivudine, although no longer recommended by the WHO due to increased risk of toxicity, continues to be used in a significant proportion of patients in resource-limited regions because of its low cost.

In patients receiving regimens with a high genetic barrier to resistance, such as those recommended by the DHHS guidelines, the most common reasons for virological failure are nonadherence and, potentially, pharmacokinetic factors or minority transmitted drug-resistant variants. In resource-limited settings, additional challenges include intermittent lack of access to ARVs, fewer ARV choices if toxicity or intolerance arises, and incomplete virological suppression in patients with high plasma HIV-1 RNA levels who are treated with regimens having lower genetic barriers to resistance.<sup>[166]</sup> Genotypic resistance testing provides essential information because it can indicate whether virological failure was due primarily to non-adherence (i.e. if no drug resistance mutations are detected) and can also be used to help guide

second-line therapy. Before selecting a second-line ART regimen, it is essential to determine the factors most likely responsible for the failure of the initial ART regimen.

In patients developing virological failure on first-line ART, the extent of drug resistance is roughly proportional to the duration of uncontrolled virus replication in the face of selected drug pressure. In resource-limited countries where patients undergo infrequent virological monitoring, samples from patients with virological failure generally contain more drug-resistance mutations and higher levels of cross-resistance than virological samples from patients with virological failure in well resourced regions.<sup>[167]</sup>

In tables II and III, we summarize the most common drug-resistance patterns associated with the most frequently used DHHS- and WHO-recommended first-line therapies, and provide guidance on selecting second-line therapy based upon these patterns. Table II describes drug-resistance patterns seen with commonly used NRTI ‘backbones’. If M184V alone is present (the most common scenario in closely monitored patients), tenofovir/lamivudine or tenofovir/emtricitabine should probably be used as part of second-line

therapy whether or not tenofovir was used in the first regimen. Zidovudine/lamivudine is an acceptable alternative. If TAMs are present, as often occurs in patients receiving a zidovudine- or stavudine-containing regimen, a tenofovir-containing regimen is preferred for second-line therapy. If K65R is present, as occasionally occurs in patients receiving a tenofovir- or stavudine-containing regimen, zidovudine/lamivudine is preferred for second-line therapy. Table III describes drug-resistance patterns seen with commonly used non-NRTI components (i.e. the ‘base’) of a first-line regimen. This table underscores the important role of boosted PIs in second-line therapy, even in cases where the initial ARV regimen contained a boosted PI.

## 8. Salvage Therapy

The principles of salvage therapy for patients for whom more than one regimen has failed are similar to those for patients for whom a single regimen has failed: the salvage regimen should be sufficiently potent to suppress virus levels to below the level of detection, and should have a sufficiently high genetic barrier to resistance to prevent virological rebound. Therefore, a salvage

**Table II.** Genotypic resistance profiles associated with dual NRTIs used for initial ART: implications for choice of NRTIs for second-line ART<sup>a</sup>

NRTIs	Early mutations	Late mutations <sup>b</sup>	Recommended second-line regimen <sup>c</sup>
TDF/FTC	M184V	K65R > K70E	Continue TDF/FTC unless K65R or K70E are present, in which case use AZT/3TC
TDF/3TC	M184V, K65R	K65R > K70E	Continue TDF/3TC or switch to TDF/FTC unless K65R or K70E are present, in which case use AZT/3TC
ABC/3TC	M184V	L74V > K65R, Y115F	Change to TDF/FTC unless K65R or Y115F are present, in which case use AZT/3TC
AZT/3TC	M184V	TAMS >> Q151M, T69ins	Change to TDF/FTC
d4T/3TC	M184V	TAMS > K65R >> Q151M, T69ins	Change to TDF/FTC if TAMs are present Change to AZT/3TC if K65R is present

a Refer to table I for a full list of drug name abbreviations and definitions.

b High-level multi-NRTI resistance associated with the Q151M complex or the T69 insertion has been observed almost exclusively with AZT/3TC and d4T/3TC (or older ddl-containing regimens). Whether this association is due to the drug combinations themselves or due to the fact that these dual NRTIs were used in regions with infrequent laboratory monitoring is not known. TAMs: M41L, D67N, K70R, L210W, T215YF and K219QE.

c ABC is a generally inferior choice for salvage compared with TDF because M184V decreases susceptibility to ABC but increases susceptibility to TDF. For patients at high risk for advanced NRTI resistance and few available treatment options, some experts have advocated the use of TDF/FTC/AZT or AZT/3TC/ABC.

**ARV** = antiretroviral therapy; **NRTI** = nucleoside reverse transcriptase inhibitor; **TAMs** = thymidine-analogue associated mutations; > indicates occurs more frequently than; >> indicates occurs much more frequently than.

**Table III.** Genotypic resistance profiles associated with initial antiretroviral therapy<sup>a</sup> failure: implications for choice of second-line antiretroviral therapy

First-line therapy		Early mutations <sup>b</sup>	Later mutations <sup>b</sup>	Strategy for second-line therapy		
Base	Backbone			NRTIs <sup>c</sup>	Recommended base <sup>d</sup>	Alternative base <sup>d</sup>
EFV	2 NRTIs	M184V and/or 1–2 NNRTI DRMs	Additional NRTI and NNRTI DRMs	2 NRTIs	DRV/r	ATV/r or LPV/r > RAL or MVC
NVP	2 NRTIs	M184V and/or 1–2 NNRTI DRMs	Additional NRTI and NNRTI DRMs	2 NRTIs	DRV/r	ATV/r or LPV/r > RAL or MVC
ATV/r	2 NRTIs	M184V	Additional NRTI DRMs Rarely ATV/r DRMs	2 NRTIs	DRV/r	LPV/r or DRV/r or RAL > MVC
LPV/r	2 NRTIs	M184V	Additional NRTI DRMs Rarely LPV/r DRMs	2 NRTIs	DRV/r	RAL > MVC
DRV/r	2 NRTIs	M184V	Additional NRTI DRMs	2 NRTIs	DRV/r or RAL	RAL > MVC
RAL	2 NRTIs	M184V± 1–2 RAL DRMs	Additional NRTI and RAL DRMs	2 NRTIs	DRV/r	LPV/r or ATV/r > ETR or EFV or MVC

a Refer to table I for a full list of drug name abbreviations and definitions.

b The most common major ARV resistance mutations for the first-line base ARVs include EFV-K103N, G190S, Y188L and V106M; NVP-Y181C, K103N, G190A/S, Y188L and V106A/M; ATV/r-I50L and N88S, LPV/r-M46I, I54V, V82A and L76V; RAL-Q148HRK, N155H and Y143CR. Of note, DRV/r-resistance mutations have not been reported to occur in patients receiving DRV/r plus two NRTIs and the ATV/r-resistance mutations do not confer LPV/r or DRV/r cross-resistance.

c Refer to table II for NRTI choices for second-line ART.

d In patients with extensive NRTI resistance and high plasma HIV-1 RNA levels, a potent boosted-PI (such as DRV/r or LPV/r) in combination with two NRTIs is the best choice for second-line therapy. If using a less potent boosted-PI, RAL, MVC, EFV or ETR in combination with two NRTIs, the addition of 1–2 previously unused ARVs should be considered.

**ART** = ARV therapy; **ARV** = antiretroviral; **DRMs** = drug-resistant mutations; **NRTI** = nucleoside reverse transcriptase inhibitor; **PI** = protease inhibitor.

regimen should optimally have three fully active ARVs.<sup>[28]</sup> In patients with complicated treatment histories, genotypic and/or phenotypic susceptibility testing is often not sufficient to identify drug-resistant variants that emerged during past therapies and may still pose a threat to a new regimen. Therefore, a thorough examination of the patient's ARV history and prior resistance tests should be performed.<sup>[168–170]</sup> Phenotypic testing is also often helpful in patients with advanced resistance, particularly for assessing susceptibility to PIs and etravirine, as the mutational patterns associated with resistance to these ARVs can be difficult to interpret.<sup>[28]</sup>

The number of available fully active ARVs is often diminished with each successive treatment failure. Therefore, a salvage regimen is likely to be more complicated in that it may require multiple ARVs with partial residual activity and compromised genetic barriers of resistance to attain complete virological suppression. The ARVs most frequently used for advanced salvage therapy

are darunavir/r, etravirine, raltegravir, maraviroc, the NRTIs and occasionally enfuvirtide and tipranavir/r. Salvage therapy ARVs can be placed into the following hierarchy: (i) ARVs belonging to a previously unused drug class; (ii) ARVs belonging to a previously used drug class but that maintain significant residual antiviral activity; (iii) NRTI combinations, as these often appear to retain *in vivo* virological activity even in the presence of reduced *in vitro* NRTI susceptibility; and (iv) ARVs associated with previous virological failure and drug resistance that appear to have possibly regained their activity as a result of viral reversion to wild type.

### 8.1 Previously Unused ARV Class

The most frequently used ARVs in this category include the integrase inhibitors,<sup>[171]</sup> maraviroc<sup>[100]</sup> and enfuvirtide.<sup>[172]</sup> Two new ARV classes are optimally recommended in building a salvage regimen, but one new class combined with fully or partially



active ARVs from previously used classes can often be sufficient for attaining sustained virological suppression. This has been exemplified in the TRIO study, in which 86% of patients with prior NRTI, NNRTI and PI experience achieved virological suppression at 48 weeks when treated with raltegravir (an ARV belonging to a previously unused class) plus etravirine and darunavir/r (new ARVs from previously used ARV classes) in combination with an optimized background regimen.<sup>[173]</sup>

### 8.2 ARVs Belonging to Previously Used Drug Classes

Boosted PIs are an important component to salvage therapy due to their high genetic barrier to resistance and incomplete intra-class cross-resistance.<sup>[77,79,174]</sup> For example, the signature drug-resistance mutations associated with atazanavir/r, I50L and N88S do not confer resistance to other PIs, and I50L confers increased susceptibility to other PIs.<sup>[175,176]</sup> Atazanavir/r is frequently used in NNRTI-experienced PI-naïve patients, but not in PI-experienced patients because the drug-resistance mutations selected by other PIs cause cross-resistance with atazanavir/r.<sup>[78,177]</sup> Thus, the PIs used most frequently in salvage therapy are lopinavir/r (particularly in resource-limited settings), darunavir/r and, less commonly, tipranavir/r.

Lopinavir/r is useful in salvage therapy because it has a high genetic barrier to resistance and often retains antiviral activity against many viruses from patients developing virological failure while receiving other PIs. However, it has a lower genetic barrier to resistance than darunavir/r and is less effective than darunavir/r at treating patients with previous PI experience.<sup>[174,178,179]</sup> Virological failure to lopinavir/r is associated with the emergence of viruses having two distinct but occasionally overlapping mutational pathways: one that retains sensitivity to darunavir/r (characterized by the development of M46I, I54V, V82A) versus one that confers darunavir/r cross-resistance (characterized by development of L76V and less commonly V32I, I47A and I50V).<sup>[180]</sup> Darunavir/r is usually the most active PI against viruses that have developed resistance to other PIs.<sup>[181]</sup> Indeed, darunavir/r-resistant viruses are generally

resistant to all other PIs, with the occasional exception of tipranavir/r.<sup>[76,79]</sup> There are some key genotypic mutations that can help identify those viruses for which tipranavir/r may be more active than darunavir/r: I50V, I54L and L76V.<sup>[77]</sup> However, genotypic resistance to tipranavir/r is not well defined, so a phenotype should ideally be obtained prior to using tipranavir/r for salvage therapy.

Etravirine is the only NNRTI recommended for salvage therapy in nevirapine- or efavirenz-resistant patients. If genotypic testing reveals few etravirine-associated drug resistance mutations, etravirine may be an important adjunct for salvage therapy. However, the immediate use of etravirine following failure of another NNRTI, without the inclusion of at least one new class of medication, should be considered cautiously, particularly if the previous NNRTI failure was associated with prolonged virus replication in the presence of NNRTI-selection pressure. This scenario increases the likelihood that a patient may harbour multiple minority variant NNRTI-resistant virus lineages, including some that may be associated with high-level etravirine resistance.<sup>[72,182]</sup>

Dolutegravir is likely to be the only INI recommended for salvage therapy in INI-experienced patients. In the VIKING I and II trials, 21/27 (78%) and 23/24 (96%) of subjects with pre-existing INI mutations administered dolutegravir once-daily (VIKING I) and twice-daily (VIKING II) as functional monotherapy achieved the primary endpoint of HIV-1 RNA <400 copies/mL ( $n = 13$ ) or  $\geq 0.7 \log_{10}$  copies/mL decline.<sup>[93]</sup> Dolutegravir has recently been made available through an expanded access programme to patients with raltegravir or elvitegravir resistance, for whom a fully suppressive ART regimen cannot be constructed with commercially available medications.<sup>[183]</sup>

### 8.3 NRTI Combinations

NRTIs appear to retain virological activity even against highly NRTI-resistant viruses, potentially as a result of the fitness costs of NRTI resistance.<sup>[41,184]</sup> For this reason NRTIs, in particular lamivudine or emtricitabine, are often maintained in salvage regimens because of partial activity

or their effect on viral fitness, but it is not clear whether this is beneficial in cases of severe NRTI resistance.<sup>[185,186]</sup> This issue is currently under investigation by the AIDS Clinical Trials Group (ACTG)-sponsored OPTIONS (The Optimized Treatment that Includes or Omits NRTIs) trial.<sup>[187]</sup>

#### 8.4 Reuse of ARVs That Were Previously Associated with Virological Failure and Drug Resistance

In situations where the number of fully or partially active ARVs from the first three categories in this section is insufficient to create a fully suppressive regimen, reuse of a previously resistant ARV can be considered if genotypic or phenotypic testing has documented that the dominant circulating virus population has reverted to a drug-susceptible wild-type form.<sup>[188]</sup> Depending on the time since treatment discontinuation, there will be a risk that low-level resistant circulating variants or chromosomally integrated resistant virus variants will greatly shorten the efficacy of a previously used ARV. However, such an ARV may provide essential antiviral activity during the period of greatest risk for a salvage therapy regimen – when the virus load is at its highest.

Due to the complexity of managing highly treatment-experienced patients, it is recommended that, in cases of highly resistant virus, an expert HIV clinician be consulted. As we have discussed in the previous sections, the most potent ARVs for the treatment of patients with advanced resistance are those from new ARV classes, followed by ARVs from pre-existing classes that are not affected by cross-resistance. Unfortunately, with the possible exception of dolutegravir, there are few ARVs in the drug development pipeline that are likely to have a major impact on salvage therapy for the next few years. Thus, understanding and optimizing current ARV resources for patients requiring salvage therapy is of the utmost importance.

## 9. Summary

Understanding of HIV drug resistance is critical in order to tailor effective antiviral regimens for in-

dividuals and populations. When thinking about drug resistance and future ARV options, clinicians need to consider the patient's likely adherence and access to ARVs as well as drug interactions, tolerability, prior ARV history and the results of current and prior genotypic or phenotypic resistance testing. Important concepts to consider when constructing salvage regimens are those of ARV potency, genetic barrier to resistance and cross-resistance. In resource-limited settings where genotypic resistance testing is not widely available, surveillance of primary resistance and the development of low-cost resistance assays are of utmost importance. Regardless of the setting, understanding the basic principles of HIV drug resistance is helpful in guiding individual clinical decisions and the development of ARV treatment guidelines.

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- Correspondence: Dr *Michele W. Tang*, M.D., Stanford University, Division of Infectious Diseases, 300 Pasteur Drive, Grant Building, S101D, Stanford, CA 94305-5107, USA  
E-mail: [micheletang@gmail.com](mailto:micheletang@gmail.com)