Antiviral Research 85 (2010) 245–265

Contents lists available at ScienceDirect

Antiviral Research

journal homepage: www.elsevier.com/locate/antiviral

Review

Clinical management of HIV-1 resistance

Roger Paredes*, Bonaventura Clotet

Institut de Recerca de la SIDA – irsiCaixa & Fundació Lluita contra la SIDA, Servei de Medicina Interna, Hospital Universitari Germans Trias i Pujol, Universitat Autònoma de Barcelona, Catalonia, Spain

ARTICLE INFO

Article history:
Received 6 September 2009
Accepted 30 September 2009

Keywords:
Human Immunodeficiency Virus
Antiretroviral resistance
Antiretroviral treatment
Genotype
Phenotype
Ultrasensitive resistance testing
Allele-specific PCR
Ultradeep sequencing

ABSTRACT

Antiretroviral drug resistance is a fundamental survival strategy for the virus that stems from its vast capacity to generate diversity. With the recent availability of new ARV drugs and classes, it is now possible to prescribe fully active ART to most HIV-infected subjects and achieve viral suppression even in those with multidrug-resistant HIV. It is uncertain, however, if this scenario will endure. Given that ART must be given for life, and new compounds other than second-generation integrase inhibitors may not reach the clinic soon, all efforts must be done to avoid the development of resistance to the new agents. Here, we discuss relevant aspects for the clinical management of antiretroviral drug resistance, leaving detailed explanations of mechanisms and mutation patterns to other articles in this issue.

This article forms part of a special issue of Antiviral Research marking the 25th anniversary of antiretroviral drug discovery and development, vol. 85, issue 1, 2010.

Contents

* Corresponding author at: Institut de Recerca de la SIDA – irsiCaixa, Servei de Medicina Interna, Hospital Universitari Germans Trias i Pujol, Universitat Autònoma de Barcelona, Crta. de Canyet s/n, Planta 2a, 08916 Badalona, Catalonia, Spain. Tel.: +34 93 465 6374x162; fax: +34 93 465 3968.
E-mail address: rparedes@irsicaixa.es (R. Paredes).

0166-3542/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.antiviral.2009.09.015

1. Introduction

The primary goals of antiretroviral therapy (ART) are to reduce HIV-related morbidity, prolong survival, improve quality of life, restore and preserve immunologic function and prevent HIV transmission (Hammer et al., 2008). By combining at least three antiretroviral (ARV) drugs, ART is able to suppress viral replication to below 50 copies/mL during several years (Riddler et al., 2008). This usually results in immune restoration, increased survival and better quality of life.

Antiretroviral drug resistance is a fundamental survival strategy for the virus that stems from its vast capacity to generate diversity. Antiretroviral resistance pre-dates therapy initiation, it can be generated spontaneously or be acquired from the source of infection and evolves in every subject, albeit at different rates depending on the virological efficacy of therapy. Pre-existing or evolving ARV drug resistance hampers the clinical benefits of ART. Studies show that antiretroviral drug resistance testing is cost-effective (Sax et al., 2005), and improves the virologic, immunologic and clinical outcomes of ART (Baxter et al., 2000; Harrigan et al., 1999; Kuritzkes et al., 2008; Tural et al., 2002; Vray et al., 2003). With the recent availability of new ARV drugs and classes, it is now possible to prescribe fully active ART to most HIV-infected subjects and achieve viral suppression even in those with multidrug-resistant (MDR) HIV. It is uncertain, however, how long this scenario will endure, given that ART must be given for life and new compounds in the pipeline may not reach the clinic soon.

Here, we discuss relevant aspects for the clinical management of antiretroviral drug resistance, leaving detailed explanations of mechanisms and mutation patterns to other articles in this issue (cross-reference to other articles in this issue of AVR).

2. General principles of antiretroviral resistance

2.1. Resistance as a consequence of HIV diversity

Antiretroviral drug resistance may be defined as the need of increasing concentrations of antiretrovirals to suppress HIV replication relative to non-resistant viruses. Viral susceptibility is expressed as the drug concentration able to inhibit virus growth *in vitro* to 50% (50% inhibitory concentration, IC_{50}) or 90% (IC_{90}), relative to a wild-type reference virus (Hirsch et al., 2008). Replication of partially resistant HIV can be reduced by increased drug concentrations; therefore, antiretroviral drug resistance is a *continuum*. As an exception, phenotypic resistance to CCR5 antagonists is characterized by lack of inhibitory activity despite increasing concentrations of drug, which is shown as decreased in percent maximal inhibition rather than shifts in IC_{50}.

In order to manage HIV resistance properly, clinicians must keep in mind that HIV has a quasispecies distribution in every infected individual (Domingo and Holland, 1997). This implies that circulating viruses are not represented by a unique virus genotype, but by a swarm of different but genetically related viral variants. This viral population structure is consequence of an error-prone reverse transcriptase (RT) that lacks proofreading activity and engages in frequent strand transfers during reverse transcription, leading to frequent recombination events, alongside the presence of high rates of virus replication (Domingo and Holland, 1997; Coffin, 1995) (Table 1). Extant models of virus replication predict that any single mutant and many double mutants can be generated daily through viral replication; conversely, few triple mutants, if any, should predate therapy in subjects infected with non-resistant HIV-1. Each viral variant in the quasispecies has a different replicative capacity in different environments (Quinones-Mateu and Arts, 2002). The wild-type (WT) variant is the one with better ability to replicate in the absence of therapy and, therefore, predominates in untreated subjects. Suboptimal treatment reduces replication of the WT and may select for mutants with a fitness advantage in the presence of therapy. Selection of mutants occurs at a rate proportional to the level of replication and the relative fitness advantage in that particular environment. Persistent viremia under ART leads to further accumulation of mutations, which increase resistance or improve viral fitness. Because resistance mutations usually confer a fitness cost to the virus in the absence of therapy, ART interruption usually leads to a relatively fast decay of mutants and re-emergence of the WT virus. Such mutants remain incorporated in the viral quasispecies and viral reservoirs as minority variants that can re-emerge if ART selective pressure is exerted again (Le et al., 2009; Metzner et al., 2003). By generating escape mutants *a priori* and storing evolved resistant variants, the viral quasispecies is able to overcome subsequent immunologic or pharmacologic pressure.

Combined ART tackles HIV's adaptative mechanisms by taking advantage of: (a) the unlikely pre-existence of viral variants resistant to more than 3 drugs in treatment-naïve subjects (Coffin, 1995), (b) the tight association between the level of viral replication and the rate of viral evolution (i.e. viral evolution is greatly reduced in aviremic subjects), (c) the higher flexibility or increased affinity of new antiretrovirals to bind virus targets with resistance mutations, and (d) the possibility of achieving increased drug levels at target sites through pharmacokinetic enhancement with ritonavir or other compounds.

2.2. Replication, genetic barrier, adherence and resistance

The likelihood of developing antiretroviral resistance depends on the relative potency of the antiretroviral regimen and the degree of ongoing replication in the presence of therapy (Coffin, 1995; Condra et al., 1995; Domingo and Holland, 1997; Flexner, 1998; Kuritzkes et al., 1996; Molla et al., 1996; Tisdale et al., 1995) (Fig. 1). A regimen with low antiviral potency creates a minimal selective pressure to the virus and leads to slow resistance evolution, even if replication persists. A more potent regimen that is unable to suppress viral replication leads to an increased selective pressure over the virus, which rapidly accumulates resistance. Finally, a highly potent regimen that decreases viral replication to minimal levels is associated with slow resistance accumulation, despite the potent selective pressure exerted to the virus.

Each ARV drug has a different genetic barrier, i.e. number of mutations required, to attain resistance. The nucleoside reverse transcriptase inhibitors (NRTIs) lamivudine and emtricitabine, the non-nucleoside reverse transcriptase inhibitors (NNRTIs)

Table 1
Features of HIV replication that explain HIV's ability to diversify.

10^{9-12} new virions are generated every day.
10^{-3} to 10^{-4} mutations (one or two per genome) are spontaneously generated per replication cycle.
3–4 recombination events are produced per replication cycle.

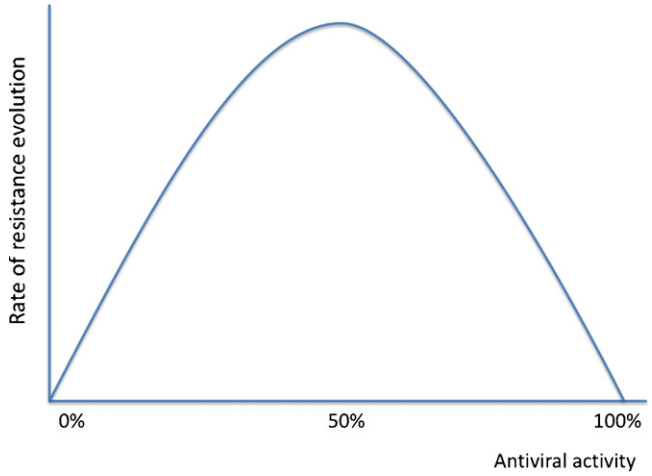

Fig. 1. Relation between antiviral drug activity and emergence of resistance. *Source*: Pillay D, Zambon M., 1998. Education and debate: Antiviral drug resistance. BMJ 317, 660–662.

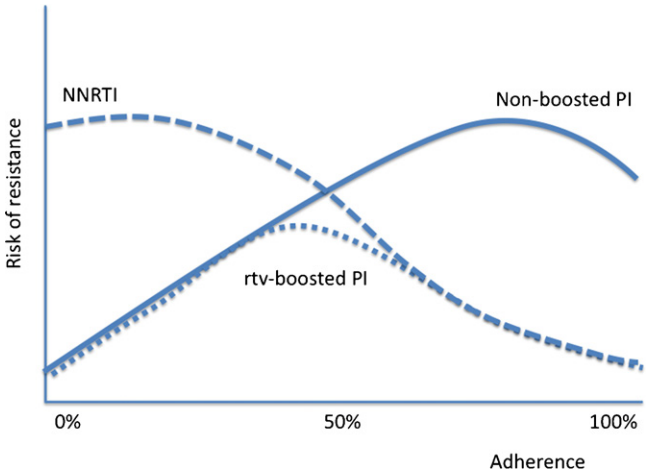

Fig. 2. Relationship between medication adherence and the risk of developing PI or NNRTI drug resistance. Resistance to single PI therapy occurs most frequently at moderate to high levels of adherence, resistance to NNRTI therapy occurs at low to moderate levels of adherence, and resistance to ritonavir-boosted PI therapy is most likely to occur at middle ranges of adherence. PI, Protease inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor.
Source: Bangsberg DR, Moss AR, Deeks SG, 2004. Paradoxes of adherence and drug resistance to HIV antiretroviral therapy. J. Antimicrob. Chemother. 53, 696–699.

nevirapine, efavirenz, and delavirdine, and the fusion inhibitor (FI) enfuvirtide require only one resistance mutation to attain high-level phenotypic resistance, being considered 'drugs with low genetic barrier'. Ritonavir-boosted protease inhibitors (PIs) and the NRTIs zidovudine and stavudine require the accumulation of several mutations to achieve high-level resistance. Drugs with 'intermediate genetic barrier' include the integrase strand-transfer inhibitors (InSTIs) raltegravir and elvitegravir, the second-generation NNRTI etravirine and the NRTIs didanosine, tenofovir and abacavir. The genetic barrier to small-molecule CCR5 antagonists is uncertain; clinically, most subjects developing virological failure to these drugs actually show emergence of pre-existing, often undetected, X4 viruses rather than genotypic changes in envelope leading to stereotypical resistance (Tsibris et al., 2009; Westby et al., 2006).

Suboptimal ART adherence is associated with increased risk of virological failure and development of ARV resistance (Nachega et al., 2007, 2005; Schackman et al., 2007). Each antiretroviral therapeutic class has a unique adherence–resistance relationship (Fig. 2) (Bangsberg et al., 2004). NNRTI-treated individuals rarely develop resistance at high levels of adherence due to the virological effectiveness of these regimens. NNRTI resistance develops rapidly at moderate to low levels of resistance due to the low 'fitness' costs associated with single mutations. Single PI-treated individuals may develop resistance at high levels of adherence because residual viral replication is often seen in such patients. PI resistance is uncommon at low levels of adherence because of the significant fitness costs associated with these mutations. Resistance to a ritonavir-boosted PI is only possible in a narrow range of adherence where there is sufficient drug around to select for mutations that reduce fitness while still allowing residual viral replication.

2.3. Clinical consequences of antiretroviral resistance

The immediate consequence of ARV resistance is loss of treatment efficacy. Given the molecular structure similarities within compounds of the same antiretroviral family and their interaction with similar target sites, the emergence of resistance to one drug often leads to cross-resistance to other drugs of the same family. This reduces the therapeutic arsenal available for salvage therapy leading to the prescription of more complex, expensive and often worse tolerated regimens (Table 2).

Resistance-associated virological failure creates a 'vicious circle' where ARV options are reduced, consecutive treatment lines are associated with progressively reduced duration of antiviral efficacy and each new virological failure is associated with further resistance accumulation (Fig. 3). Subjects entering this vicious circle often end up developing viruses with reduced susceptibility to all drug classes.

Certain mutations conferring high-level resistance to one agent may increase viral susceptibility to another compound, resulting in a so-called "hypersusceptible" virus to the other agent. For example, mutation I50L in protease confers resistance to atazanavir but increases susceptibility to tipranavir and other PIs (Colonno et al., 2004). In addition, many resistance-conferring mutations decrease replication capacity in comparison with the WT virus. The clinical correlates of mutation-derived "hypersusceptibility" and replication capacity measurements, however, remain largely unknown.

Three independent studies found that the emergence of antiretroviral resistance among patients starting first-line ART was associated with a nearly 2-fold increased risk of death (Hogg et al., 2006; Kozal et al., 2007; Lohse et al., 2007). Interestingly, emergence of resistance to NNRTIs was associated with a greater risk of subsequent death (3-fold increase) than resistance to any other drug class.

Importantly, development of drug resistance also increases the probability of transmission of drug-resistant viruses from person-

Table 2
Consequences of antiretroviral resistance[a].

Loss of treatment efficacy
Cross-resistance
Increased mortality
Need to prescribe ART that is:
More complex
More toxic
More expensive
Shorter duration of antiviral efficacy of subsequent ART
Increased risk of resistance evolution under subsequent ART
Some mutations, hypersusceptibility to certain ARVs[b]
In general, reduced viral fitness relative to WT[b]
Risk of transmission of resistant HIV

[a] ART: Antiretroviral therapy; ARV: antiretroviral; WT: wild-type.
[b] Clinical significance uncertain.

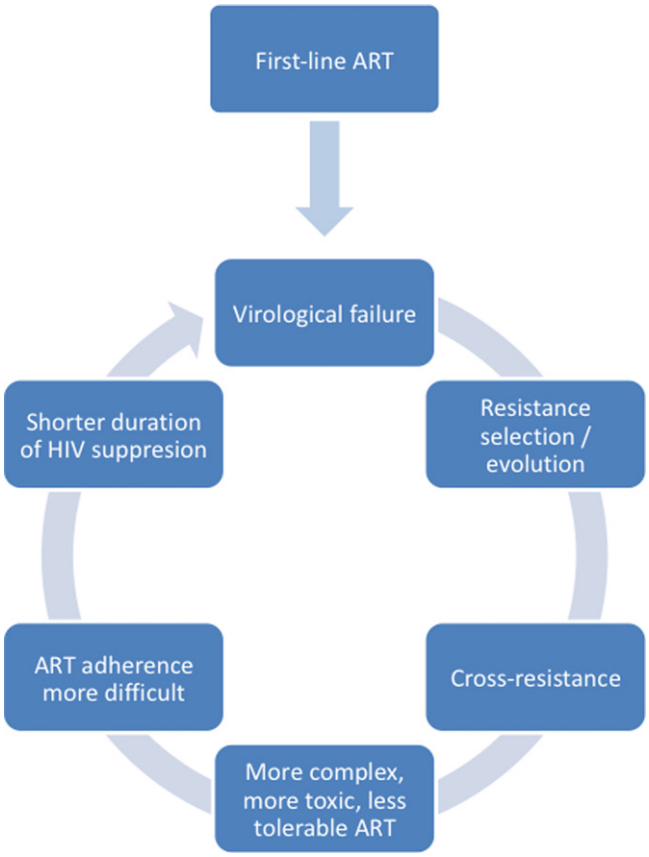

Fig. 3. 'Vicious circle' of resistance-associated virological failure.

to-person as primary infections or superinfection particularly if suppression of viremia becomes unfeasible in the source subject due to ARV resistance.

3. Clinical management of antiretroviral resistance

3.1. Objectives

Antiretroviral drug resistance is both an individual and a public health problem: clinicians must address both levels simultaneously. At the individual level, clinicians must seek to maximize the potency and durability of the antiviral activity of ART by providing patients with ARV regimens to which the virus retains maximum susceptibility. At the public health level, clinicians must seek to reduce the incidence and prevalence of antiretroviral resistance in the society, so that more individuals retain fully susceptible viruses, less of them transmit resistant viruses, and more can be effectively treated when needed.

3.2. Management principles

Resistance-associated virological failure must be prevented by (Hirsch et al., 2008): (a) identifying pre-existing or primary resistance in antiretroviral naïve subjects; (b) tailoring first-line ART to ensure that all components of the drug regimen retain full antiretroviral activity; (c) maximizing the antiviral potency of the regimen alongside its tolerability and convenience in order to ensure adequate long-term adherence; (d) detecting virological failure early and identifying emerging resistance mutations in order to select the most active treatment combinations, and (e) managing virological failure aggressively, i.e. switching early to a new ARV regimen tailored according to drug resistance testing, preferably including drugs with high genetic barrier to attain resistance.

These individual-based measures should exert public health benefits by: (a) decreasing HIV transmission overall, because more subjects would remain aviremic, and (b) decreasing transmission of ARV-resistant HIV, given that fewer resistant viruses should circulate among human populations with adequate access to ART. How ARV drug resistance will evolve in countries scaling up ARV remains uncertain.

3.3. Identifying antiretroviral resistance

The goal of resistance testing is to provide information to assist in the selection of the antiretroviral regimen(s) more likely achieve and maintain viral suppression.

All guidelines agree that HIV drug resistance testing should be performed when HIV-infected persons enter clinical care, whether or not they will be treated immediately (Hirsch et al., 2008) (Table 3). This strategy attempts to maximize the chances of detecting transmitted drug resistance. In those individuals in whom treatment is deferred, resistance testing should be repeated before therapy initiation. In addition, genotypic resistance testing is recommended for all pregnant women prior to initiation of therapy and for those entering pregnancy with detectable HIV RNA levels while on therapy.

In HIV-infected individuals receiving antiretroviral therapy, resistance testing should be performed in the presence of virological failure. To ensure adequate performance of resistance testing, HIV-1 RNA levels should be at least 1000 copies/mL at the time of testing, although guidelines agree that resistance testing could also be attempted in individuals with HIV-1 RNA levels between 500 and 1000 copies/mL. In this last group of patients, however, the chances of amplifying HIV-1 sequences are markedly lower.

Treatment guidelines also suggest that drug resistance testing might also be helpful when managing suboptimal viral load reduction (Hirsch et al., 2008). This is less clear, however, because the addition of, or switch to, new antiretroviral drugs could be very helpful to achieve viral suppression in this situation regardless resistance testing results.

Importantly, given that drug resistance mutations wane after treatment interruption (Castagna et al., 2006; Deeks et al., 2003, 2005, 2001; Martinez-Picado et al., 2002; Metzner et al., 2003; Paredes et al., 2009; Walter et al., 2002), drug resistance testing in the setting of virologic failure should be performed while the patient is taking his/her antiretroviral drugs, or within 4 weeks after discontinuing therapy.

3.4. Tropism testing

Guidelines agree that co-receptor tropism assays should be performed whenever the use of a CCR5 inhibitor is being considered (Hirsch et al., 2008). In addition, co-receptor tropism testing might be considered for patients who exhibit virologic failure on CCR5 antagonist containing regimens because virological failure to CCR5 antagonists is frequently associated with a CCR5 to CXCR4 tropism switch. Given that that the emergence of X4 viruses is associated with accelerated progression towards AIDS or death (Koot et al., 1993), co-receptor tropism testing has been proposed as a tool to guide initiation of antiretroviral therapy or to establish prognosis. However, these indications are not supported by current guidelines because studies addressing these questions specifically are lacking. It is also unclear whether upfront co-receptor testing in subjects initiating first-line antiretroviral therapy would be useful in case a CCR5 antagonist would be required later on (e.g. in case of toxicity to the initial treatment). Viral tropism switch could occur, even if subjects with sustained viremia suppression.

Table 3
Summary of clinical situations in which resistance testing is recommended (IAS-USA, July 2008).

Clinical setting	Comments
Before initiation of therapy	
Primary (acute and early) infection	Resistance testing is recommended. Initial therapy may be altered based on resistance test results.
First evaluation of chronic HIV-1 infection	Resistance testing is recommended, including for patients for whom therapy is delayed, because plasma wild-type isolates may replace drug-resistant virus with time in the absence of treatment.
Treatment initiation for chronic HIV-1 infection	Resistance testing is recommended because of a rising prevalence of baseline HIV-1 drug resistance in untreated patients with chronic infection, unless pre-existing data or stored samples for testing are available.
In antiretroviral-treated patients	
Treatment failure	Resistance testing is recommended. The decision to change therapy should integrate treatment history, new and prior resistance results (if available), and evaluation of adherence and possible drug interactions.
In specific settings	
Pregnancy[a]	Resistance testing is recommended before initiation of therapy to effectively treat the mother and prevent mother-to-child transmission.
Other considerations and general recommendations	Postexposure prophylaxis should consider treatment history and resistance data from the source, when available; A sudden increase in HIV-1 plasma RNA may reflect superinfection, possibly with drug-resistant virus; Plasma samples to be tested for drug resistance should contain at least 500 HIV-1 RNA copies/mL to ensure successful PCR amplification required for all sequencing approaches; It is preferable that the blood sample for resistance testing be obtained while the patient is receiving the failing regimen, if possible; Resistance testing should be performed by laboratories that have appropriate operator training, certification, and periodic proficiency assurance; Genotypic and phenotypic test results should be interpreted by individuals knowledgeable in antiretroviral therapy and drug resistance patterns; Inhibitory quotient testing is not recommended for clinical decision-making.

Source: Hirsch et al. (2008).
[a] If resistance test results are available before the pregnancy, clinical judgment should guide whether retesting for resistance is necessary.

3.5. Interpreting resistance testing results

Drug resistance testing interpretation is a difficult task. Genotypic data used to infer phenotypic susceptibility *in vivo* is fragmentary. HIV genotypes obtained through viral population sequencing are consensus sequences that do not capture infrequent viral variants (i.e. those at levels below 15–20% of the viral population). In addition, genotypic analyses frequently focus on discrete regions of the HIV genome, although missing substitutions in outside regions may also modulate resistance or viral fitness (e.g. connection and RNAse H domains of RT or PR cleavage sites in Gag or Gag-Pol) (Delviks-Frankenberry et al., 2007; Lastere et al., 2004; Yap et al., 2007). Importantly, different mutations may interact between each other or have a different effect on drugs from the same family; indeed, some mutations conferring resistance to particular drugs may increase susceptibility to others. Such limitations may force clinicians to infer phenotypic effects from complex genotypes based on weak evidence-based data. Efforts are constantly underway to develop and refine treatment interpretation rules. Such rules can be based on expert opinion, which has the potential for information biases, or on complex mathematical algorithms and machine-learning methods, where computer programs extract rules from comparisons between genotypes paired with phenotypes or with clinical efficacy data. Fortunately, a fair amount of clinically relevant information can be extracted from this complex picture. Moreover, incorporating such information into the clinical management of HIV-infected patients clearly improves their chances to do well on therapy (Hirsch et al., 2008). Tables 4 and 5 outline the basic mechanisms of resistance and the principal aspects of resistance interpretation relevant for clinical management, respectively. Readers may find more detailed information on this regard in other chapters of this issue.

3.6. Management of primary antiretroviral resistance

The clinical management goal for ARV resistance in treatment-naïve subjects is to ensure durable virological suppression with first-line ART by identifying pre-existing ARV resistance and avoiding prescription of regimens to which the virus is not fully susceptible (Fig. 3).

3.6.1. Origin of primary resistance

Resistant viruses can either be spontaneously generated or transmitted from person-to-person through contact with blood or blood products, sexual intercourse or from mother-to-child (Little, 2000; Little et al., 1999). Resistant viruses are transmitted less efficiently than wild-type (Leigh Brown et al., 2003), although multidrug-resistant variants are sometimes transmitted (Little, 2000; Little et al., 2002; Richman et al., 2004; Wensing et al., 2005). Interestingly, only a few variants present in the "donor" viral population are able to establish primary HIV infection even if transmission occurs through direct blood-to-blood contact. This suggests a transmission bottleneck, the nature of which remains poorly understood. Because resistant variants are often transmitted alone, the viral population in the recipient subject is almost exclusively conformed by resistant viruses, which remain predominant until wild-type revertants are generated through spontaneous mutation (Little et al., 2008).

In the absence of drug pressure and lack of more fit competing viral variants, fixation of new mutants or revertants is a slow process (Bonhoeffer et al., 2002). In a longitudinal follow-up of 14 recently HIV-infected patients with transmitted drug-resistant virus (Little et al., 2008), the median time to loss of detectable drug resistance using population-based assays ranged from 4.1 years to longer than the lifetime of the individual.

Conversely, resistant mutants generated through replication errors often co-exist and compete with the WT in the quasispecies. As a result, mutants often become extinct or, sometimes, persist in the viral quasispecies at very low frequency, as predicted from the Poisson distribution. Whereas transmitted resistant variants can contain several resistance mutations in various genes, mutants generated spontaneously in the absence of ART pressure hardly ever accumulate more than 2 resistance-associated substitutions in the same genome.

Table 4
Principal mechanisms of antiretroviral drug resistance[a].

Drug family	Mechanism of action	Mechanism of resistance
NRTIs and NtRTIs	NRTIs and NtRTIs are chain terminators. They are incorporated into the nascent chain of viral DNA. Because they lack a 3′ hydroxyl group, no additional nucleotides can be appended.	Impaired nucleotide incorporation: M184V, K65R and the Q151M complex selectively impair RT's ability to incorporate an analogue into DNA. Nucleotide excision: TAMs allow ATP to bind RT near the 3′ end of viral DNA terminated by the incorporation of a nucleoside analogue. ATP then excises the analogue from viral DNA, allowing reverse transcription to proceed normally.
NNRTIs	Small molecules with strong affinity for a hydrophobic pocket located near the catalytic domain of RT. Inhibitor binding affects the flexibility of the enzyme, thereby blocking its ability to synthesize DNA.	Most NNRTI resistance mutations affect residues that are directly involved in inhibitor binding. Few have been found to act indirectly, by changing the position or the orientation of the aminoacids involved with direct contact with the inhibitor. Etravirine (ETV) is a diarylpyrimidine with some conformational isomerism that can bind RT in multiple conformations, allowing for a more robust interaction between ETV and the enzyme, even in the presence of some mutations (K103N).
PIs	PIs mimic the structure of the natural viral substrates of the HIV PR, competing with them for binding in the enzyme's active site.	Mutations in direct contact with the inhibitor or of distant aminoacids that modify the overall shape of the PR cavity disrupt fitting of the PI within the cavity and induce resistance.
InSTI	Raltegravir and Elvitegravir are DNA strand-transfer inhibitors that block the joining of the processed viral DNA ends into the host chromosome.	Binding requires divalent metal and resistance is metal dependent with active site mutants displaying resistance only when the enzymes are evaluated in the context of Mg(2+). There is extensive cross-resistance between the two drugs.
Fusion inhibitors (enfuvirtide)	Enfuvirtide is a 36-mer synthetic oligopeptide that binds to the trimeric HR-1 complex, preventing the association of HR-1 with HR-2 and inhibiting fusion.	Changes in a conserved amino acid triad (GIV) at positions 36–38 and in amino acids 39–45 in the HR1 region of gp41 prevent ENF binding.
CCR5 antagonists	CCR5 antagonist binding alters the conformational state of the CCR5 receptor, inhibiting the binding of gp120 to CCR5 by an allosteric mechanism.	Resistant viruses acquire the ability to recognize receptor conformations stabilized by CCR5 antagonists.

[a] RT: Reverse transcriptase; PR: protease; IN: integrase; HR1: first heptad repeat; HR2: second heptad repeat; NRTI: nucleoside reverse transcriptase inhibitor; NtRTI: nucleotide reverse transcriptase inhibitor; NNRTI: non-nucleoside reverse transcriptase inhibitor; PI: protease inhibitor; InSTI: integrase strand-transfer inhibitor; NVP: nevirapine; EFV: efavirenz; ETV: etravirine; ENF: enfuvirtide.

3.6.2. *Prevalence of primary resistance*

The prevalence of primary resistance (Table 6) varies as effective ART is introduced in a given population. Resistance neither evolves nor is transmitted in populations with no ART available. Increased rates of secondary resistance in the treated population due to suboptimal therapy and/or inadequate therapeutic monitoring tend to be followed by increases in the prevalence of primary resistance. As ART becomes more potent, includes drugs with higher genetic barrier and the ART coverage of subjects in need of treatment increases, more individuals remain aviremic and fewer harbor resistant viruses at the time of treatment failure. At this point, the prevalence of primary resistance may remain stable or start to decline.

The prevalence of primary resistance in well-resourced countries ranges from 8% to 19% for any drug, 5% to 12% for NRTIs, 2% to 8% for NNRTIs and 3% to 7% for PIs (Ross et al., 2008; Shet et al., 2006; Truong et al., 2006; Wensing et al., 2005; Yerly et al., 2007) (Table 7). After a period of steady increases, the overall prevalence of primary ARV resistance seems to have stabilized around 10% in most industrialized countries. According to some reports, it could even have started to decline in certain regions in Europe. By drug class, only resistance to NNRTIs has clearly increased in the last decade, probably due to the widespread use of these compounds coupled with their low genetic barrier and the minimal impact of NNRTI resistance mutations on virus fitness. Fortunately, the prevalence of primary dual or triple-drug resistance has remained at low levels (<2%). Primary resistance to new ARV drugs like the integrase strand-transfer inhibitors (InSTIs) is virtually zero due to their recent introduction in clinical management, but needs to be monitored prospectively.

To date, rates of primary resistance have remained low in developing countries. However, the high frequency of secondary resistance in several resource-limited settings, suggests that the prevalence of primary antiretroviral resistance could increase in the coming years.

In a study conducted in Zambia, primary drug resistance mutations were observed in 5% of patients starting first-line ART (Hamers et al., 2008). Among treatment-naive patients in western India, drug resistance mutations were documented in 10% of those tested, including the V82A mutation in PR as well as the RT mutations D67N and M184V (Gupta et al., 2008). Among treatment-naive patients in southern Brazil, the presence of primary drug resistance was seen in approximately 5% of patients. The most frequent mutations were those associated with NNRTI resistance, particularly the K103N mutation, which was observed in 4.3% of patients (Rodrigues et al., 2008). In other countries like Mali (year 2006) (Derache et al., 2008) and Tanzania (year 2005) (Nyombi et al., 2008), the prevalence of primary resistance remained below 5%.

3.6.3. *Antiretroviral resistance is underestimated by viral population genotypic assays*

Current viral population sequencing assays only detect viral variants present in more than 15–20% of the quasispecies (Brun-Vezinet et al., 2004; Hirsch et al., 2008). New genotypic assays like allele-specific PCR (ASPCR), single-genome sequencing (SGS), LigAmp or ultradeep 454 sequencing (UDS), enable detection of minority HIV-1 resistant variants at levels $\leq$1% (Cai et al., 2007; Charpentier et al., 2004; Flys et al., 2007; Johnson et al., 2005, 2008; Le et al., 2009; Loubser et al., 2006; Metzner et al., 2005; Palmer et al., 2006; Paredes et al., 2007b; Simen et al., 2009). Several primary resistance surveillance studies using ultrasensitive resistance assays have demonstrated increases in detection of HIV-1 variants with primary resistance of at least 2–3-fold relative to standard population-based sequencing (Metzner et al., 2005; Paredes et al.,

Table 5
Main features of antiretroviral drug resistance relevant for clinical management[a].

Drug class	Mechanism	Main mutation or pathway	Drugs	Effects	References
NRTIs	Impaired nucleotide incorporation	M184I/V	HLRes: 3TC, FTC	Signature mutations for 3TC and FTC: confer > 1000-fold resistance to these drugs	(Back et al., 1996; Boucher et al., 1993; Descamps et al., 2000; Deval et al., 2004; Diallo et al., 2003; Frost et al., 2000; Gao et al., 1993; Gulick et al., 2004; Havlir et al., 2000; Huang et al., 1998; Ji and Loeb, 1994; Katlama et al., 1996; Kuritzkes et al., 1996; Miller et al., 1998; Schinazi et al., 1993; Schuurman et al., 1995; Sharma and Crumpacker, 1999; Tisdale et al., 1993; Wainberg, 2004; Wainberg et al., 1996, 1995)
			PRes: ABC and ddI	Also confer partial resistance to ABC and ddI	
			HS: ZDV and TDF	Generate hypersensitivity to and synergy with ZDV and TDF M184V is associated with 0.5 log reduction in HIV-1 RNA levels during 3TC therapy Increased RT processivity and multiple other effects M184V emerges rapidly with single or dual 3TC exposure and is the most frequent mutation in 3-drug ART M184I emerges first due to RT's mutational bias towards G → A replacements, but M184V is fitter than WT and M184I in the presence of 3TC and subsequently replaces them	
		K65R	PRes: TDF, ddI, ABC	Signature mutation for TDF: confers 2-fold resistance to TDF	(Garcia-Lerma et al., 2003; Gu et al., 1995a, 1994, 1995b; Miller et al., 2001; Moyle, 2004; Nikolenko et al., 2004; Roge et al., 2003; Shah et al., 2000; Winston and Stebbing, 2004)
			3TC, FTC, d4T	Intermediate resistance to ddI, ABC, 3TC, FTC	
			HS: ZDV	Low-level resistance to d4T Increased susceptibility to ZDV; does not develop in patients receiving ZDV-containing regimens	
		L74V	HLRes: ddI	Signature mutation for ddI: confers ~2-fold resistance to ddI, being sufficient to cause VF in subjects under ddI monotherapy	(Miller et al., 2000; Ray et al., 2002)
			PRes: ABC	Intermediate resistance to ABC: VF requires accumulation of additional mutations	
			HS: ZDV and TDF	Increased susceptibility to ZDV and, less clearly, to d4T and TDF	
		Q151M complex	Multi NRTI resistance	Selected during d4T or ddI failure or after prolonged ZDV exposure	(Johnson et al., 2006; Naugler et al., 2002; Sirivichayakul et al., 2003; Vandamme et al., 2004)
			PRes: ZDV, ddI, d4T, and ABC, 3TC, FTC and TDF	Intermediate resistance to ZDV, ddI, d4T, and ABC Low-level resistance to 3TC, FTC Overall, no resistance to TDF, but isolates with V75I + F77L + F116Y + Q151M → low-level 3TC and TDF resistance Seen in <5% of HIV strains with NRTI resistance, often alongside mutations at positions 62, 75, 77, and 116, which increase resistance and restore RC	
		69 insertion complex	Multi NRTI resistance	Only in 2% of heavily pre-treated patients and confer high-level resistance to TDF and other NRTIs	
		T69N/S/A	Multi NRTI resistance	Most frequent substitutions other than TAMs and M184V, contributes to resistance to every NRTI	

Table 5 (*Continued*)

Drug class	Mechanism	Main mutation or pathway	Drugs	Effects	References
		V75T	Multi NRTI resistance	Resistance to d4T, ddI and ddC, generally in the context of multinucleoside resistance	
	Nucleotide excision	Thymidine analogue resistance mutations (TAM)	HLRes: ZDV and d4T	Resistance to ZDV and d4T, and partially to ABC, ddI and TDF	(Balzarini et al., 1993; Boucher et al., 1992a, 1992b; Calderon et al., 1995; Johnson et al., 2006; Larder et al., 1989, 1991; Larder and Kemp, 1989; Miller and Larder, 2001; Miller et al., 1998; Quinones-Mateu and Arts, 2002; Richman, 1990; St Clair et al., 1991; Tisdale et al., 1991; Vandamme et al., 2004)
			PRes: ABC, ddI and TDF	Two TAM pathways that tend to be mutually excluding, TAM-1: 41L, 210W, 215Y. → Stronger clinical cross-resistance to TDF than TAM-2 in subtype B viruses TAM-2: 67N, 70R and 219E/Q → Less TDF cross-resistance Significant decreases in NRTI susceptibility require the stepwise accumulation of several resistance mutations, which increases cross-resistance TAM-1: 215Y → 41L + 215Y → 41L, 210W, 215Y TAM-2: 70R → 67N + 70R → 67N + 70R + 219 E/Q	
NNRTIs	Change in the hydrophobic binding site that prevents effective drug binding	Overall	First generation NNRTIs: NVP and EFV	NVP and EFV have low genetic barrier to attain resistance	(Hsiou et al., 2001; Johnson et al., 2006; Picchio et al., 2008; Ren et al., 2001; Richman et al., 1994; Vandamme et al., 2004; Wirden et al., 2003)
				Three spatial clusters around the NNRTI binding pocket: Cluster 1 (p66): L100I, K103N, V106A and V108I Cluster 2 (p66): Y181C, Y188L/C/H, and G190S/A Cluster 3 (p51): P225H, M230L, and P236L Extensive phenotypic and clinical cross-resistance to NVP and EFV Minimal impact on HIV's replication capacity	
		Y181C	HLRes: NVP Pres: EFV	Frequent in NVP failure Confers high-level resistance to NVP but only 2-fold resistance to EFV Increases HIV-1 susceptibility to ZDV → selected against during NVP failure in the presence of ZDV, leading to the emergence of K103N variants	
		K103N	HLRes: EFV and NVP	First resistance mutation to appear during EFV failure High-level EFV and NVP resistance	
		Overall	2nd generation NNRTIs: ETV	More flexible structure: Higher genetic barrier than NVP or EFV TAMs and M184V increase susceptibility to ETV *in vitro*	
		K103N ETV RAMs: V90I, A98G, L100I, K101E/H/P, V106I, E138A, V179D/F/T, Y181C/I/V, G190S/A and M230L		No effect on ETV susceptibility Weighted classification based on clinical outcome in the DUET trials: 3 Y181I/V 2.5 K101P, L100I, Y181C, M230L 1.5 E138A, V106I, G190S, V179F* 1 V90I, V179D/T, K101E/H, A98G, G190A	(Peeters et al., 2008; Vingerhoets et al., 2008)

			Interpretation: Weighted mutation score 0–2 2.5–3.5 = or >4 *comparable to the control arm	Response rates in DUET trials 74% (highest response) 52% (intermediate response) 38% (reduced response)*	
NRTI and NNRTI	Alteration of the dimerization of p66/p51 heterodimers or retardation in RNAseH processivity	Connection and RNAseH domain mutations	Pres: ZDV, d4T, 3TC, ABC and TDF	Overall, increase ZDV resistance when combined with TAMs	(Gupta et al., 2006a, b; Yap et al., 2007)
			Some, PRes to NVP and/or EFV	Also confer resistance to 3TC, ABC and TDF, but have little effect on ddI or d4T Usually absent in ARV-naïve, coselected with TAMs Located in genomic regions not routinely investigated by standard genotype; clinical significance unclear In addition, the following cause NNRTI resistance N348I: 7.4-fold to NVP; 2.5-fold to EFV; enhances NNRTI resistance in the presence of K103N; may appear early after ART failure T369I: 3-fold to EFV; 2.3-fold to ZDV A376S: 5-fold to NVP; no resistance to NVP E399G: 3.6-fold to EFV; reduces RC in the presence of L100I, V106I, V179D, and F227C None of these had an effect on clinical response to ETV in the DUET trials	
PIs	Disruption of PI fitting within the PR catalytic cavity	G48V, L90M, I84V	SQV	Signature SQV RAMs: G48V and L90M. Each, 3- to 10-fold resistance; both >100-fold resistance G48V occurs first *in vitro*, but L90M is the most frequent *in vivo* L90M reduces susceptibility to most other PIs, particularly NFV *In vivo*, G48V frequently occurs with V82A → cross-PI resistance I84V is often observed alongside L90M; i84v + l90M decreases susceptibility 10–20-fold Accessory mutations: L24I, M46I/L/V, V82A/T/F/S HS to SQV conferred by I50L and L76V	(Ermolieff et al., 1997; Schapiro et al., 1999; Winters et al., 1998)
		M46I, V82A/F/T, I84V	IDV	Signature IDV RAMs: M46I, V82A/F/T, and I84V. Muts at positions 10, 20, 24, 54, 63, 64, 71, and 90 in PR also associated with resistance *in vivo*; mutation # predicts degree of resistance Ordered appearance M46L/I and V82A → I54V or A71V/T. Cross-resistance to RTV, APV, LPV/r, and TPV; also to SQV, NFV, and ATV. M46I, L63P, V82T, and I84V: resistance to most PIs	(Condra et al., 1995; Zhang et al., 1997)

Table 5 (*Continued*)

Drug class	Mechanism	Main mutation or pathway	Drugs	Effects	References
		D30N, L90M	NFV	Signature RAMs: Subtype B: D30N, Non-B subtypes: L90M Ordered appearance in subtype B: D30N → L90M Accessory RAMs: M36I, M46I, A71V, N88S Cross-resistance: D30N: no cross-resistance L90M: SQV, RTV, IDV, TPV, APV, ATV, LPV/r N88S: hypersusceptibility to APV	(Devereux et al., 2001; Resch et al., 2002; Zachary et al., 2001)
		I50V	APV/FAPV	FAPV prodrug of APV (active metabolite): same resistance patterns	(Bally et al., 2000; de Meyer et al., 2008; Gulnik et al., 1995; Kempf et al., 2001; Klabe et al., 1998; Lam and Parkin, 2003; Lastere et al., 2004; Maguire et al., 2002; Marcelin et al., 2004, 2003; Martinez-Picado et al., 2005; Monno et al., 2003; Shafer et al., 1998; Yanchunas et al., 2005)
		I54L/M and V32I + I47V I84V		Signature RAM: I50V 2–3-fold DS to APV, but ↓ RC → additional substitutions in Gag often required Greatest DS to DRV susceptibility, but ↑ susceptibility to TPV. I54L/M and V32I + I47V Frequent in RTV-boosted APV or FAPV, but they usually appear in patients with further experience to PIs I54L/M and V32I + I47V reduce the virologic response to ritonavir-amprenavirand confer cross-resistance to other PIs I84V Less common than I50V, I54L/M, or V32I + I47V DS to LPV, IDV, NFV, RTV, SQV and TPV V82F + I84V further DS to RTV, IDV, NFV, and APV Accessory RAMs: positions 10, 32, 46, 47, 54, 73, and 90 Hypersusceptibility to APV: K20T and N88S and I50L	
		M46I/L, I54V/T/A/S, and V82A/T/F/S	LPV/rtv	Two pathways:	(Carrillo et al., 1998; Delaugerre et al., 2007; Friend et al., 2004; Grant et al., 2008; Kagan et al., 2005; Kempf et al., 2001; King et al., 2007; Masquelier et al., 2002; Mo et al., 2005; Nijhuis et al., 2007; Parkin et al., 2003; Prado et al., 2002; Winters et al., 2008)
				IDV-like: M46I/L, I54V/T/A/S, and V82A/T/F/S.	
		V32I, I47V/A, I50V, I54L/M and L76V		APV-like: V32I, I47V/A, I50V, I54L/M and L76V. Accessory mutations: L24I and F53L RAMs selected by other PIs decreasing LPV susceptibility: G48V, I84V I84A/C, L90M	
		I50L, I84V, I54L	ATV	Signature RAM: I50L Frequent when ATV is used without RTV as the initial PI, Less frequent with ATV/rtv or in previously PI-treated patients. Significant DS to ATV but increased susceptibility to other PIs In initial PI failure, ATV may select for A71V + I50L: ↑ ATV resistance but ↓ RC In PI-experienced subjects or when combined with SQV, atazanavir can select for I84V and, less frequently, for I54L Cross-resistance: I50L: no cross-resistance to other PIs I84V: broad cross-resistance I54L: major DRV resistance mutation Other PI RAMs V82A and L90M confer cross-resistance to ATV	(Colonno et al., 2004, 2003)

	V32I, I47V, I54V/A/M, V82L/T and I84V	TPV	V82L/T and I84V Most common RAMs during TPV/rtv salvage therapy Mutations V82L/T are associated with the greatest decreases in TPV susceptibility *in vitro* I47V, I54A/V/S/M, I84V: also associated with DS to TPV Accessory RAMs: L33F/I, E35G, K43T, Q58E, T74P, N83D TPV hypersusceptibility conferred by: L24I, I50V, I50L, I54L, and L76V Weighted classification of tipranavir mutations (RESIST trials) Class / Weight / Mutations and weight of each mutation ↑ response / <0 / 24I (−2), 50L/V (−4), 54L (−7), 76V (−2) Minor RAMs / 1–2 / 10V (+1), 36I (+2), 43T (+2), 46L (+1), 84V (+2) Major RAMs / >2 / 47V (+6), 54A/M/V (+3), 58E (+5), 74P (+6), 82L/T (+5), 83D (+4) Interpretation: Weighted mutation score / Interpretation based on RESIST trials < or = 3 / Susceptible >3 and ≤10 / Partially susceptible >10 / Resistant	(Baxter et al., 2006; Hicks et al., 2006; Naeger and Struble, 2007; Sherer, 2007)
	V11I, V32I, L33F, I47V, I50V, I54L/M, G73S, L76V, I84V, and L89V	DRV	Weight classification of DRV mutations based on fold-change in IC_{50} *in vitro* Fold-change / Mutations >4 / 50V 3–4 / 54M, 76V, 84V 2–3 / 32I, 33F, 47V, 74P* <2 / 11I, 54L, 89V In some studies, mutation 74P is associated with a fold-change comparable to that of I50V. Interpretation: diminished response to DRV when 3 or more of these mutations are present at baseline V82F: does not develop in viruses from patients receiving DRV/r, but has a major effect on DRV/r susceptibility V82A: presence of V82A in patients with 3 DRV RAMs is associated with a virological response comparable to that observed in patients with 2 DRV RAMs		(de Meyer et al., 2008; Pellegrin et al., 2008; van Marck et al., 2007)
InSTI	Disruption of the functional sequentration exerted by InSTIs of the Mg^{2+} cofactor in the enzyme's active site	Q148H/K/R	RAL	Extensive cross-class resistance with ELV	(Hazuda et al., 2007)
		N155H		3 apparently mutually excluding pathways defined by Q148H/K/R, N155H and Y143C/R	
		Y143C/R		10–25-fold DS but impaired RC Q148H: greatest reduction in RC Accessory mutations (G140S in the presence of Q148H) compensate RC losses, or to further decrease susceptibility to RAL Secondary mutations: L74M, E92Q, T97A, E138K, V151I, G163G/R, and D232D/N Only modest DS but add DS to N155H or Q148R/H/K N155H pathway associated with: L64M, E92Q, V151I, T97A, G163K Q148K/R/H pathways associated with: G140A/S and E138K Y143C/R pathway: being characterized Often *in vivo*: N155H → replaced by Q148H → fitness cost compensated by Q148H + G140S Mutations Q148H/K/R and N155H also confer resistance to elvitegravir (ELV)		

Table 5 (*Continued*)

Drug class	Mechanism	Main mutation or pathway	Drugs	Effects	References
		E92Q E138K Q148R/K/H N155H	ELV	E92Q: signature mutation for ELV Most frequent mutations after ELV failure: E92Q, E138K, Q148R/K/H and N155H (each in 39% of subjects) S147G (32%) T66I/A/K (18%)	(McColl et al., 2007)
Fusion inhibitors	Change in the 3D structure of HR-1 (and sometime HR2) that disables T-20 binding	G36D/S, V38A, Q40H, N42T/E/S, N43D/S/K, L44M, L45M	ENF	Single substitutions in HR1: 5–10-fold DS Double mutants: G36S + L44M, N42T + N43K/S, V38A + N42T/D, V38E + N42S → Highest levels of resistance Other viral factors (e.g. the V3 loop, or the HR2 region) may modulate the sensitivity of the gp41 36–45 amino acid core region Mutations in HR2 (N126K and S138A), can also contribute to T20 resistance Mutations in HR1 and HR2 confer high fitness cost	(Derdeyn et al., 2000; Greenberg and Cammack, 2004; Rimsky et al., 1998; Wei et al., 2002; Xu et al., 2005)
CCR5 antagonists	Structural changes in env (V3-loop and others) improve fusion efficiency and viral entry even in receptor conformations stabilized by MRV or VCV	MRV, VCV		The dominant pathway to VF to CCR5 antagonists *in vivo* is a shift from CCR5 to CXCR4 use Most resistant viruses have mutations in the V3-loop stem, which are sufficient to confer VCV resistance The effect of resistance mutations depends on the remaining envelope context. Thus, resistance to CCR5 antagonists may not result in stereotypical mutations Phenotypic resistance is evidenced by decreases in the percent maximal inhibition in resistant viruses relative to control, instead of right shifts in the IC_{50} curves Resistance mutations confer a fitness cost to the virus in the absence of drug The extent of cross-resistance within the class is not yet known	(Fatkenheuer et al., 2008; Gulick et al., 2007; Saag et al.; Tsibris and Kuritzkes, 2007; Tsibris et al., 2008; Westby et al., 2006; Westby et al., 2007)

[a] HLRes: High-level resistance; PRes: partial resistance; HS: hypersusceptibility, DS: decreased susceptibility; IC_{50}: 50% inhibitory concentration; RAM: resistance-associated mutation; RC: replication capacity; VF: virological failure; VR: virological response; RT: reverse transcriptase; PR: protease; IN: integrase; HR1: first heptad repeat; HR2: second heptad repeat; NRTI: nucleoside reverse transcriptase inhibitor; NtRTI: nucleotide reverse transcriptase inhibitor; NNRTI: non-nucleoside reverse transcriptase inhibitor; PI: protease inhibitor; InSTI: integrase strand-transfer inhibitor; ZDV: zidovudine; ddI: didanosine; ddC: zalcitabine; d4T: stavudine; ABC: abacavir; TDF: tenofovir; 3TC: lamivudine; FTC: emtricitabine; NVP: nevirapine; EFV: efavirenz; ETV: etravirine; rtv: tritonavir; SQV: saquinavir; IDV: indinavir; NFV: nelfinavir; ATV: atazanavir; APV: amprenavir; FAPV: fosamprenavir; LPV: lopinavir; DRV: darunavir; TPV: tipranavir; RAL: raltegravir; ELV: elvitegravir; ENF: enfuvirtide; MRV: maraviroc; VCV: vicriviroc.

Table 6
Factors influencing the prevalence of primary resistance in a given population.

Epidemiological
Prevalence of acquired (secondary) HIV resistance in the population
Fraction of subjects on ART who remain viremic
ART coverage of the population in need of treatment
Characteristics (particularly, genetic barrier to attain resistance) of the drug regimens given to the population
Existence of HIV transmission "hotspots", i.e. clusters of individuals frequently engaging in high-risk transmission practices
Biological
Mutational bias towards G to A replacements during viral replication
Fitness cost of resistance mutations, which influences their detectability in plasma
Persistence of transmitted mutants though 'compensatory fixation'
Technical or methodological
Sensitivity of the assay used to detect resistance
Definition of ARV resistance
Sample selection and representativity

2007a). However, the ability of ultrasensitive resistance testing to help design more effective ARV therapy needs to be further elucidated before such assays can be systematically implemented in the surveillance of primary ARV resistance.

3.6.4. Clinical implications of primary resistance

There is strong evidence that subjects with primary NNRTI resistance detected by viral population sequencing are at a higher risk of virological failure. In a case-cohort study of the ACTG A5095 Trial, for example, the risk of virologic failure to initial efavirenz-containing ART for subjects with primary NNRTI resistance by standard genotype was higher than that for subjects without such resistance (hazard ratio 2.27 [95% confidence interval], 1.15–4.49; $P = .018$) (Kuritzkes et al., 2008).

Studies addressing the effect of primary resistance on virological response overall or to other ARV regimens have shown more conflicting results (Bannister et al., 2008; Pillay et al., 2006). This is not surprising, considering that the correlation between primary resistance and therapeutic response is influenced by the knowledge of baseline resistance patterns at the time of initiation of therapy, the availability of alternative treatment options and by the ability of prescribed drugs to suppress replication of viruses with intermediate resistance.

Most studies agree that presence of transmitted drug resistance is associated with longer time to virological suppression under first-line ART (Grant et al., 2002; Little et al., 2002; Peuchant et al., 2008). However, although time to virological failure was shorter in the presence of baseline resistance in studies performed in the early 2000s, recent large cohort studies have failed to detect this association. Interestingly, one study from the CASCADE collaboration reported a steeper CD4+ count decline in subjects with primary resistance than in those without, but only during the first year after infection (Pillay et al., 2006).

Cohort studies may not necessarily be the best setting to detect differences on ART outcome according to primary resistance. Clinicians use ARV resistance information to design ART; inadequate control of this factor could potentially lead to information biases. Also, cohort studies may not adequately capture the evolution of ARV resistance interpretations over time and may not always consider resistance data within the adequate ART groups. These factors could potentially dilute significant associations. On the other hand, prospective randomized trials addressing the impact of primary resistance on current ART outcomes may face important ethical issues and may not be available to solve these questions. Another explanation for the lack of association in recent studies is that more and better alternative ART approaches to which the virus remains fully susceptible are now available.

The fact that treatment initiation at earlier calendar periods but not the number of active drugs impacted first-line treatment efficacy likely reflects that, in the context of primary resistance, having multiple alternatives is not necessarily better than having a few good ones. In other words, to achieve an adequate response to initial ART, the number of good options available is not as important as not making the wrong choice.

3.6.5. Recommendations for the clinical management of primary resistance

To date, first-line ART with efavirenz and lamivudine (two drugs with low genetic barrier) plus another NRTI is the ART option more resilient to virological failure (Riddler et al., 2008). This shows, first, that drugs with higher genetic barrier do not necessarily lead to higher treatment success rates and, second, that antiretroviral resistance is only one relevant factor influencing ART outcome: tolerance, convenience, toxicity, and adherence are equally important aspects to maximize the success of first-line ART. Another important factor is to plan for second and future ART lines in the event of virological failure. In this regard, virological failure to first-line NNRTI-based ART is more frequently associated with detection of resistance to NRTIs and to the "third regimen" than failure to boosted-PI regimens.

Thereby, given the high number of alternatives for first-line ART, clinicians should use ARV resistance information to:

(a) Design ART combinations that incorporate drugs to which the virus is fully susceptible.
(b) In the infrequent event of transmission of MDR HIV:
  i. Investigate the resistance profile of the source, if available.
  ii. Maximize the predicted antiviral activity of the regimen. If partial antiviral activity is expected, it seems reasonable to prioritize the inclusion of drugs with high genetic barrier.
(c) Plan for the best second and further ART lines in the event of treatment failure.
(d) In every case, ART design should integrate resistance information alongside considerations regarding tolerance, convenience, and toxicity of regimens, as well as the patient's ability to adhere to ART in the long-term.

3.7. Management of acquired or secondary ARV resistance

The goal of ARV resistance management in treatment-experienced subjects is to minimize as much as possible the accumulation of resistance mutations during virological failure by detecting failure as soon as possible and withdrawing the selective pressure of the failing ART, and to regain virological suppression <50 copies/mL as soon as possible with a new ARV regimen. The ultimate objectives of these actions are three-fold: (a) to preserve treatment options at the time of virological failure to the greatest extent, (b) to regain and maintain viral suppression below 50 copies/mL for the longest time possible and (c) to prevent the development of MDR HIV (Fig. 3). A public health goal derived from these individual-based objectives is to reduce the prevalence and prevent transmission of drug-resistant HIV in the population.

3.7.1. Prevalence of secondary ARV resistance

Most subjects with virological failure to ART harbor viruses with resistance mutations. This can limit the efficacy of second-line and salvage regimens, although with new drug classes, options have improved. Today, however, fewer subjects show resistance mutations at the time of virological failure than one decade ago. In statistically rigorous analyses, the prevalence of drug resistance in therapy-exposed subjects was estimated to be 50–60% in 1999, and decreased to 39–53% in 2006 (vonWyl et al., 2008). The prevalence of triple-drug-resistant virus remained stable at 5%. Fortunately,

Table 7
Prevalence of antiretroviral resistance mutations in US ART-naïve HIV-infected patients, 2001–2007 (Ross et al.).

	2001		2007	
	IAS	Stanford	IAS	Stanford
Major resistance mutations	9%	5%	20%	13%
NRTI	3%	4%	4%	5%
NNRTI	6%	2%	15%	8%
PI	2%	2%	3%	3%
Dual class	2%	2%	2%	2%
Triple class	<1%	<1%	<1%	<1%

In a large cohort of 3542 ART-naïve HIV-infected patients from 36 US states and District of Columbia enrolling into clinical trials between 2001 and 2007, the prevalence of primary resistance evolved increased from 2001 to 2007, mainly at the expense of the prevalence of primary NNRTI resistance. IAS: International AIDS Association, USA; Stanford: Stanford HIV Drug Resistance Database.

extensive virological failure of the three veteran classes of drugs occurs slowly in routine clinical practice. Of note, initial therapy with boosted PI-based regimens results in less resistance within and across drug classes. In a systematic review of clinical trials of adults receiving first-line ART, which consisted of dual NRTIs combined with either an NNRTI or a ritonavir-boosted PI, virological failure rates at week 48 were comparable, but the incidence of M184V and K65R mutations in RT, as well as resistance to the third agent were higher for subjects starting NNRTI-based ART (Table 8).

Less information exists about the prevalence of acquired resistance in developing countries. In a recent evaluation by the South African Resistance Cohort Study (Marconi et al., 2008) the overall prevalence of resistance mutations in individuals failing their first HAART regimen in KwaZulu Natal was higher than 80%. Of 115 individuals recruited in the study, 62 (54%) were receiving d4T + 3TC + NNRTI; 43 (38%) were treated with ZDV + 3TC + NNRTI; 5 (4%) were receiving 2 NRTI + LPV/rtv, and 5 (4%) were being treated with other ARV combinations. The prevalence of dual class resistance (essentially due to the detection of the M184V mutation plus at least one NNRTI resistance mutation) was higher than 60%. The most common mutation detected at the time of virological failure was M184V/I (64.3%); K103N was present in 51.3% and V106M in 19.1%. Thymidine analogue resistance mutations (TAM) were found in 32% of subjects, with a predominance of the TAM-2 over the TAM-1 pattern (TAM-2: 19%; TAM-1: 7%; both TAM-1 and 2: 6%). The K65R mutation was only found in 3 subjects (2.6%) and each K70E and L74V mutations were detected in only 2 subjects (1.7%).

Similarly, genotypic resistance testing in 98 HIV-1-infected patients from Thailand who experienced treatment failure with their first antiretroviral regimen (a fixed-dose combination of stavudine, lamivudine, and nevirapine) during 2003–2005 showed a prevalence of at least one major NRTI or NNRTI mutation of 95% and 92%, respectively. M184V was observed in 89% of patients. TAMs, K65R, and Q151M were observed in 37%, 6%, and 8% of patients, respectively (Sungkanuparph et al., 2007).

3.7.2. Rates of resistance accumulation during virological failure

Maintenance of ARV therapy in subjects with virological failure leads to further resistance accumulation, cross-resistance and loss of treatment options. In an analysis of 106 chronically HIV-infected patients on stable antiretroviral regimen for at least 120 days but with a plasma HIV RNA level above 1000 copies per mL and at least one genotypic resistance mutation (Hatano et al., 2006), the risk of losing one fully suppressive drug or two partly suppressive drugs was estimated to be 32% at 1 year. The risk of developing a new nucleoside-associated mutation at 1 year was 23%, and the risk of developing a new major protease mutation was 17%. Similarly, an EuroSIDA study found that in patients kept on the same virologically failing ART regimen for a median of 6 months, there was considerable accumulation of drug resistance mutations, particularly in patients with initial low level of resistance to the failing regimen (Cozzi-Lepri et al., 2007). Clinicians must prevent resistance accumulation by detecting virological failure early, and quickly switching ART to fully suppressive combinations.

3.7.3. Clinical implications of acquired resistance

As shown in Fig. 3, resistance-associated virological failure is an important complication of ART that reduces the chances of adequate clinical management of HIV-infected subjects and is associated with increased morbidity and mortality. It constraints subsequent ART options for the patient, forces clinicians to prescribe more complex and sometimes worse tolerated regimens. This, in turn, makes treatment adherence more difficult and increases the chances of subsequent virological failure. If resistance-associated virological failure is not adequately managed, patients may develop viruses with resistance to several or even all drug classes. From a public health perspective, subjects with drug-resistant viruses are less likely to remain aviremic and, therefore, are at a higher risk of transmitting HIV infection, also viruses carrying drug resistance. In addition, drug resistance increases the complexity and cost of ART.

3.7.4. Recommendations for clinical management of acquired resistance

The management of acquired resistance should be based on the following points:

(a) When designing ART for the first time, clinicians should plan for potential ART schemes to be used in the event of virological failure to the first ART line.
(b) All efforts should be undertaken to diagnose virological failure early.
(c) Genotypic testing should be ordered as soon as virological failure is detected. All expert laboratories should be able to produce genotypic testing data from plasmas with HIV-1 RNA levels

Table 8
Incidence of mutations conferring resistance to key drugs (NRTIs, NNRTIs, or bPIs) at week 48. Meta-analysis including 20 clinical trials that comprised 30 treatment arms and 7970 patients.

	Initial ART with NNRTIs + 2 NRTIs	Initial ART with ritonavir-boosted PIs + 2 NRTIs	*P* value
% of virological failure at week 48	4.9% (95% CI, 3.9–6.1%)	5.3% (95% CI, 4.4–6.4%)	0.50
% M184V	35.3% (95% CI, 29.3–41.6%)	21.0% (95% CI, 14.4–28.8%)	<0.001
% K65R in subjects with no ZDV exposure	5.3% (95% CI, 2.4–9.9%)	0.0% (95% CI, 0.0–3.6%)	0.01
% resistance to the "third" agent (NNRTI or PI, respectively)	53% (95% CI, 46–60%)	0.9% (95% CI, 0.0–6.2%)	<0.001

>1000 copies/mL. If HIV-1 RNA levels are between 500 and 1000 copies/mL genotypic results can frequently be obtained when HIV-1 RNA is extracted from 1 mL of plasma after centrifugation.

(d) Salvage therapy should be initiated as soon as virological failure is confirmed. Treatment design should be based on ART history and the prediction of the most likely resistance patterns to occur.

(e) Unless the turnover time of resistance testing results is very fast, clinicians should not wait to receive genotypic testing results to switch ART: that could prolong the period of viral replication under suboptimal therapy unnecessarily and increase the chances of accumulating ARV resistance.

(f) Until resistance testing is available, clinicians should preferably prescribe drugs with high genetic barrier to resistance (e.g. protease inhibitors like darunavir/ritonavir or others, second-generation NNRTIs like etravirine and/or integrase inhibitors like raltegravir or elvitegravir) and seek for the highest antiviral activity. In settings with full availability of new ARVs, it should often be possible to design a regimen containing at least two drugs to which the virus remains fully susceptible.

(g) Resistance testing results must be used to fine-tune the salvage regimen designed; i.e. prioritize compounds to which the virus has the highest predicted susceptibility and possibly higher genetic barrier, remove drugs with small antiviral activity and, if possible, preserve active compounds for subsequent treatment lines.

3.8. Management of MDR HIV infection

Until the recent advent of new drugs and classes, between 5% and 10% of subjects in the clinic had MDR HIV and no ART options left to regain virological suppression. Where all 6-drug classes are available, it is now very rare to encounter patients in whom a potent, suppressive regimen cannot be mustered. In areas where newer drug classes and PIs are still not available, physicians encounter situations where there are no or few treatment options. Here, it might be reasonable to continue the same antiretroviral regimen until newer and presumably active agents become available in order to preserve immune responses and delay clinical progression. The major risk of this approach is ongoing viral evolution and the loss of future drug options. Alternatively, simplified approaches aiming to preserve viruses with reduced fitness can be attempted (Deeks et al., 2005). By no means should ART be interrupted in subjects without better treatment options because this is clearly associated with higher morbidity and mortality than remaining on a failing regimen (El-Sadr et al., 2006; Lawrence et al., 2003).

The question remains for how long the effects of new ARVs will persist in subjects with previous MDR HIV. Integrase strand-transfer inhibitors, for example, are drugs with an intermediate drug resistance barrier. Resistance to these drugs can develop quickly if they are not given alongside other active regimens. Given that few new drugs are expected to reach the clinic in the immediate future, it is likely that the medical management of subjects with MDR HIV will remain a major challenge in the coming years.

4. Clinical relevance of minority HIV-1 resistant variants

The clinical significance of minority resistant variants is not fully established (Table 9). Most studies show that detection of pre-existing minority NNRTI-resistant variants in ARV-naïve subjects increases the risk of virological failure to first-line NNRTI-based regimens more than 3-fold, particularly in the presence of adequate treatment adherence (Metzner et al., 2009; Paredes et al.,

Table 9
Principal techniques to detect minority HIV drug-resistant variants[a].

	Standard cloning	Single genome sequencing (SGS)	Allele-specific PCR (ASPCR)	Parallel allele-specific sequencing (PASS)	LigAmp	Ultra deep sequencing (UDS)*
Principle	Analysis of single CFUs with individual clones	Massive sequencing of single genome molecules	Differential amplification of mutants vs WT in real-time PCR	Single-base allele sequencing of polonies fixed to an acrylamide surface	Template-dependent ligation of 2 primers and quantification with Q-PCR	Massively parallel microfluidic solid-surface sequencing of single molecules
Sensitivity	Around 10% (depends on number of clones)	2%	0.003–0.4%	0.1%	0.1%	0.5–1%
# mutations	Multiple	Multiple	1	1 per round (up to 22 rounds)	1	300–400 bp
Linked mutations	Yes	Yes	No	Yes	No	Yes
Labor intensity	++	+++	+	++	+	+
Cost	+	++	+	++	+	+++
Best	Experience, PPV	Enables linkage of mutations	S, PPV, NPV, affordable	S, enables linkage of mutations	Same as ASPCR, increased specificity	Linkage, accuracy, S, NPV, rapidity of results
Worst	S, NPV	Cost, time and labor consuming	Only 1 allele 7 reaction, Sp, affected by polymorphisms	Cost, labor intensity	Same as ASPCR	Requires strong bioinformatics support, Sp

[a] PCR: Polymerase chain reaction; CFU: colony-forming units; WT: wild-type; bp: base pairs; S: sensitivity; Sp: specificity; PPV: positive predictive value; NPV: negative predictive value; Q-PCR: Quantitative PCR.
* 454 sequencing, 454 Life Sciences/Roche.

2009). These studies had a number of limitations:

(a) Study participants usually received antiretrovirals that may not be considered first choice for first-line ART (e.g. Combivir® or Trizivir®, non-boosted PIs) (Johnson et al., 2008; Simen et al., 2009).
(b) Studies were usually small, thus, they did not reach the power for predictive values.
(c) Studies were unable to define clinically relevant threshold levels of minority variants (Johnson et al., 2008; Paredes et al., 2008; Simen et al., 2009; Balduin et al., 2009; Metzner et al., 2009). One recent study, however, suggested that a total copy number of mutants >2000 copies/mL (calculated as plasma HIV-1 RNA load × proportion of mutants) was tightly associated with virological failure to first-line NNRTI-based therapy, but this has not been confirmed in other studies.
(d) Studies using allele-specific PCR (ASPCR) only assessed the effect of selected mutations (Johnson et al., 2008; Metzner et al., 2009; Peuchant et al., 2008).
(e) Studies using ultradeep sequencing (UDS) did not directly compare the ability of UDS to predict treatment failure with that of population-based sequencing (the current gold-standard in the clinic), nor did they assess mutation linkage within individual HIV genomes (Simen et al., 2009); given recent reports of frequent recombination events during pre-UDS PCR steps (Shao et al., 2009) it is unclear if assessing mutation linkage is even possible.

On the other hand, some studies did not find an increased risk of virological failure in subjects with pre-existing minority resistant HIV variants (Garcia-Lerma et al., 2003; Miller et al., 2001; Peuchant et al., 2008).

No study consistently detected an increased risk for virological failure to first-line PI-based therapy among subjects with pre-existing minority PI-resistant variants (Garcia-Lerma et al., 2003; Peuchant et al., 2008; Simen et al., 2009), suggesting that minority variants might be less relevant for patients initiating ART with high genetic barrier to resistance. On the other hand, although statistical significance was not reached, all subjects with minority PI resistance mutations in a recent UDS study ultimately experienced virological failure (Simen et al., 2009).

No study has conclusively demonstrated the clinical relevance of detecting minority resistant HIV in subjects initiating second-line or salvage regimens. However, one study found that minority HIV drug-resistant viral variants in treatment-experienced persons correlate with historical antiretroviral use (Le et al., 2009) and others suggested an association between pre-existing minority variants and virological failure in treatment-experienced patients (Kapoor et al., 2004; Lecossier et al., 2005; Roquebert et al., 2006; Svarovskaia et al., 2007).

Adequately powered studies are needed to evaluate the clinical impact of minority resistant variants to ART regimens, to compare the ability of ultrasensitive resistance technologies to predict virological failure with that of population sequencing, to define clinically relevant cut-offs for such ultrasensitive technologies, and to explore the potential for mutation linkage to improve predictions of virological outcome.

5. Summary: clinical options to prevent and manage antiretroviral drug resistance

The above considerations can be summarized in the following recommendations to prevent and manage ARV drug resistance in the clinic:

(a) Systematically screen for the presence of primary antiretroviral resistance in all subjects entering clinical care, preferably as soon after infection as possible.
(b) Adjust the design of first-line regimens to the genotypic resistance information obtained if needed. Obtaining a genotype from a subject as soon as he/she enters into clinical care may allow an increased detection of transmitted resistant viruses, which becomes harder to detect with time.
(c) Once primary resistance is ruled out, good adherence, forgiving pharmacology, potency and high genetic barrier are the principal factors associated with reduced emergence of antiretroviral resistance. Ritonavir-boosted PI-containing regimens are associated with low rates of PI resistance at treatment failure and lower rates of NRTI resistance than NNRTI-based regimens; on the other hand, first-line efavirenz-based regimens are more resilient to virological failure than ritonavir-boosted PIs tested to date, possibly due to lower compliance on PI regimens because of side effects.
(d) It is crucial to detect virological failure early and change failing therapy as soon as failure is confirmed, with the aim to re-suppress viral replication to <50 copies/mL.
(e) Use of 2 or 3 new agents is paramount to achieve durable viral suppression and prevent the future emergence of viruses with resistance to 6-drug classes.

6. Conclusions

Antiretroviral drug resistance remains a major obstacle for the adequate management of HIV infection. Adequate diagnosis and control of primary resistance, perhaps through ultrasensitive resistance testing in the near future, are paramount to maximize ART outcomes. Although the incorporation of new antiretrovirals in the anti-HIV armamentarium has improved the management of subjects infected with resistant HIV, subjects with MDR HIV remain at a high risk for developing resistance also to the new regimens. Given that new compounds in the pipeline may not reach the clinic in the immediate future, all efforts must be done to avoid the development of resistance to the new agents. Clinicians and opinion leaders should not underestimate the clinical relevance of ARV drug resistance in the past, present, and in the years to come. Whereas we are beginning to see patients with more than 20 years effective drug treatment and, currently, an important proportion of naive-patients face a totally different outcome to drug treatment, drug resistance can always emerge because of the challenges of maintaining adherence and access to chronic antiviral therapy or owing to transmitted drug-resistant viruses (Richman et al., 2009). So far, the virus always found its way to survive ART pressure.

References

Back, N.K., Nijhuis, M., Keulen, W., Boucher, C.A., Oude Essink, B.O., van Kuilenburg, A.B., van Gennip, A.H., Berkhout, B., 1996. Reduced replication of 3TC-resistant HIV-1 variants in primary cells due to a processivity defect of the reverse transcriptase enzyme. Embo J. 15, 4040–4049.
Balduin, M., Oette, M., Daumer, M.P., Hoffmann, D., Pfister, H.J., Kaiser, R., 2009. Prevalence of minor variants of HIV strains at reverse transcriptase position 103 in therapy-naive patients and their impact on the virological failure. J. Clin. Virol. 45, 34–38.
Balzarini, J., Karlsson, A., Perez-Perez, M.J., Vrang, L., Walbers, J., Zhang, H., Oberg, B., Vandamme, A.M., Camarasa, M.J., De Clercq, E., 1993. HIV-1-specific reverse transcriptase inhibitors show differential activity against HIV-1 mutant strains containing different amino acid substitutions in the reverse transcriptase. Virology 192, 246–253.
Bally, F., Martinez, R., Peters, S., Sudre, P., Telenti, A., 2000. Polymorphism of HIV type 1 gag p7/p1 and p1/p6 cleavage sites: clinical significance and implications for resistance to protease inhibitors. AIDS Res. Hum. Retroviruses 16, 1209–1213.
Bangsberg, D.R., Moss, A.R., Deeks, S.G., 2004. Paradoxes of adherence and drug resistance to HIV antiretroviral therapy. J. Antimicrob. Chemother. 53, 696–699.
Bannister, W.P., Ruiz, L., Cozzi-Lepri, A., Mocroft, A., Kirk, O., Staszewski, S., Loveday, C., Karlsson, A., Monforte, A., Clotet, B., Lundgren, J.D., 2008. Comparison of genotypic resistance profiles and virological response between patients starting nevirapine and efavirenz in EuroSIDA. Aids 22, 367–376.

Baxter, J.D., Mayers, D.L., Wentworth, D.N., Neaton, J.D., Hoover, M.L., Winters, M.A., Mannheimer, S.B., Thompson, M.A., Abrams, D.I., Brizz, B.J., Ioannidis, J.P., Merigan, T.C., 2000. A randomized study of antiretroviral management based on plasma genotypic antiretroviral resistance testing in patients failing therapy. CPCRA 046 Study Team for the Terry Beirn Community Programs for Clinical Research on AIDS. Aids 14, F83–93.

Baxter, J.D., Schapiro, J.M., Boucher, C.A., Kohlbrenner, V.M., Hall, D.B., Scherer, J.R., Mayers, D.L., 2006. Genotypic changes in human immunodeficiency virus type 1 protease associated with reduced susceptibility and virologic response to the protease inhibitor tipranavir. J. Virol. 80, 10794–10801.

Bonhoeffer, S., Barbour, A.D., De Boer, R.J., 2002. Procedures for reliable estimation of viral fitness from time-series data. Proc. Biol. Sci. 269, 1887–1893.

Boucher, C.A., Cammack, N., Schipper, P., Schuurman, R., Rouse, P., Wainberg, M.A., Cameron, J.M., 1993. High-level resistance to (−) enantiomeric 2′-deoxy-3′-thiacytidine in vitro is due to one amino acid substitution in the catalytic site of human immunodeficiency virus type 1 reverse transcriptase. Antimicrob. Agents Chemother. 37, 2231–2234.

Boucher, C.A., Lange, J.M., Miedema, F.F., Weverling, G.J., Koot, M., Mulder, J.W., Goudsmit, J., Kellam, P., Larder, B.A., Tersmette, M., 1992a. HIV-1 biological phenotype and the development of zidovudine resistance in relation to disease progression in asymptomatic individuals during treatment. Aids 6, 1259–1264.

Boucher, C.A., O'Sullivan, E., Mulder, J.W., Ramautarsing, C., Kellam, P., Darby, G., Lange, J.M., Goudsmit, J., Larder, B.A., 1992b. Ordered appearance of zidovudine resistance mutations during treatment of 18 human immunodeficiency virus-positive subjects. J. Infect. Dis. 165, 105–110.

Brun-Vezinet, F., Costagliola, D., Khaled, M.A., Calvez, V., Clavel, F., Clotet, B., Haubrich, R., Kempf, D., King, M., Kuritzkes, D., Lanier, R., Miller, M., Miller, V., Phillips, A., Pillay, D., Schapiro, J., Scott, J., Shafer, R., Zazzi, M., Zolopa, A., DeGruttola, V., 2004. Clinically validated genotype analysis: guiding principles and statistical concerns. Antivir. Ther. 9, 465–478.

Cai, F., Chen, H., Hicks, C.B., Bartlett, J.A., Zhu, J., Gao, F., 2007. Detection of minor drug-resistant populations by parallel allele-specific sequencing. Nat. Methods 4, 123–125.

Calderon, E.J., Torres, Y., Medrano, F.J., Luque, F., Larder, B., Rey, C., Sanchez-Quijano, A., Lissen, E., Leal, M., 1995. Emergence and clinical relevance of mutations associated with zidovudine resistance in asymptomatic HIV-1 infected patients. Eur. J. Clin. Microbiol. Infect. Dis. 14, 512–519.

Carrillo, A., Stewart, K.D., Sham, H.L., Norbeck, D.W., Kohlbrenner, W.E., Leonard, J.M., Kempf, D.J., Molla, A., 1998. In vitro selection and characterization of human immunodeficiency virus type 1 variants with increased resistance to ABT-378, a novel protease inhibitor. J. Virol. 72, 7532–7541.

Castagna, A., Danise, A., Menzo, S., Galli, L., Gianotti, N., Carini, E., Boeri, E., Galli, A., Cernuschi, M., Hasson, H., Clementi, M., Lazzarin, A., 2006. Lamivudine monotherapy in HIV-1-infected patients harbouring a lamivudine-resistant virus: a randomized pilot study (E-184V study). Aids 20, 795–803.

Coffin, J.M., 1995. HIV population dynamics in vivo: implications for genetic variation, pathogenesis, and therapy. Science 267, 483–489.

Colonno, R., Rose, R., McLaren, C., Thiry, A., Parkin, N., Friborg, J., 2004. Identification of I50L as the signature atazanavir (ATV)-resistance mutation in treatment-naive HIV-1-infected patients receiving ATV-containing regimens. J. Infect. Dis. 189, 1802–1810.

Colonno, R.J., Thiry, A., Limoli, K., Parkin, N., 2003. Activities of atazanavir (BMS-232632) against a large panel of human immunodeficiency virus type 1 clinical isolates resistant to one or more approved protease inhibitors. Antimicrob. Agents Chemother. 47, 1324–1333.

Condra, J.H., Schleif, W.A., Blahy, O.M., Gabryelski, L.J., Graham, D.J., Quintero, J.C., Rhodes, A., Robbins, H.L., Roth, E., Shivaprakash, M., et al., 1995. In vivo emergence of HIV-1 variants resistant to multiple protease inhibitors. Nature 374, 569–571.

Cozzi-Lepri, A., Phillips, A.N., Ruiz, L., Clotet, B., Loveday, C., Kjaer, J., Mens, H., Clumeck, N., Viksna, L., Antunes, F., Machala, L., Lundgren, J.D., 2007. Evolution of drug resistance in HIV-infected patients remaining on a virologically failing combination antiretroviral therapy regimen. Aids 21, 721–732.

Charpentier, C., Dwyer, D.E., Mammano, F., Lecossier, D., Clavel, F., Hance, A.J., 2004. Role of minority populations of human immunodeficiency virus type 1 in the evolution of viral resistance to protease inhibitors. J. Virol. 78, 4234–4247.

de Meyer, S., Vangeneugden, T., van Baelen, B., de Paepe, E., van Marck, H., Picchio, G., Lefebvre, E., de Bethune, M.P., 2008. Resistance profile of darunavir: combined 24-week results from the POWER trials. AIDS Res. Hum. Retroviruses 24, 379–388.

Deeks, S.G., Grant, R.M., Wrin, T., Paxinos, E.E., Liegler, T., Hoh, R., Martin, J.N., Petropoulos, C.J., 2003. Persistence of drug-resistant HIV-1 after a structured treatment interruption and its impact on treatment response. Aids 17, 361–370.

Deeks, S.G., Hoh, R., Neilands, T.B., Liegler, T., Aweeka, F., Petropoulos, C.J., Grant, R.M., Martin, J.N., 2005. Interruption of treatment with individual therapeutic drug classes in adults with multidrug-resistant HIV-1 infection. J. Infect. Dis. 192, 1537–1544.

Deeks, S.G., Wrin, T., Liegler, T., Hoh, R., Hayden, M., Barbour, J.D., Hellmann, N.S., Petropoulos, C.J., McCune, J.M., Hellerstein, M.K., Grant, R.M., 2001. Virologic and immunologic consequences of discontinuing combination antiretroviral-drug therapy in HIV-infected patients with detectable viremia. N. Engl. J. Med. 344, 472–480.

Delaugerre, C., Marcelin, A.G., Soulie, C., Chaix, M.L., Katlama, C., Girard, P.M., Calvez, V., Morand-Joubert, L., 2007. Transmission of multidrug-resistant HIV-1: 5 years of immunological and virological survey. Aids 21, 1365–1367.

Delviks-Frankenberry, K.A., Nikolenko, G.N., Barr, R., Pathak, V.K., 2007. Mutations in human immunodeficiency virus type 1 RNase H primer grip enhance 3′-azido-3′-deoxythymidine resistance. J. Virol. 81, 6837–6845.

Derache, A., Maiga, A.I., Traore, O., Akonde, A., Cisse, M., Jarrousse, B., Koita, V., Diarra, B., Carcelain, G., Barin, F., Pizzocolo, C., Pizarro, L., Katlama, C., Calvez, V., Marcelin, A.G., 2008. Evolution of genetic diversity and drug resistance mutations in HIV-1 among untreated patients from Mali between 2005 and 2006. J. Antimicrob. Chemother. 62, 456–463.

Derdeyn, C.A., Decker, J.M., Sfakianos, J.N., Wu, X., O'Brien, W.A., Ratner, L., Kappes, J.C., Shaw, G.M., Hunter, E., 2000. Sensitivity of human immunodeficiency virus type 1 to the fusion inhibitor T-20 is modulated by coreceptor specificity defined by the V3 loop of gp120. J. Virol. 74, 8358–8367.

Descamps, D., Flandre, P., Calvez, V., Peytavin, G., Meiffredy, V., Collin, G., Delaugerre, C., Robert-Delmas, S., Bazin, B., Aboulker, J.P., Pialoux, G., Raffi, F., Brun-Vezinet, F., 2000. Mechanisms of virologic failure in previously untreated HIV-infected patients from a trial of induction-maintenance therapy. Trilege (Agence Nationale de Recherches sur le SIDA 072) Study Team). JAMA 283, 205–211.

Deval, J., White, K.L., Miller, M.D., Parkin, N.T., Courcambeck, J., Halfon, P., Selmi, B., Boretto, J., Canard, B., 2004. Mechanistic basis for reduced viral and enzymatic fitness of HIV-1 reverse transcriptase containing both K65R and M184V mutations. J. Biol. Chem. 279, 509–516.

Devereux, H.L., Emery, V.C., Johnson, M.A., Loveday, C., 2001. Replicative fitness in vivo of HIV-1 variants with multiple drug resistance-associated mutations. J. Med. Virol. 65, 218–224.

Diallo, K., Gotte, M., Wainberg, M.A., 2003. Molecular impact of the M184V mutation in human immunodeficiency virus type 1 reverse transcriptase. Antimicrob. Agents Chemother. 47, 3377–3383.

Domingo, E., Holland, J.J., 1997. RNA virus mutations and fitness for survival. Annu. Rev. Microbiol. 51, 151–178.

El-Sadr, W.M., Lundgren, J.D., Neaton, J.D., Gordin, F., Abrams, D., Arduino, R.C., Babiker, A., Burman, W., Clumeck, N., Cohen, C.J., Cohn, D., Cooper, D., Darbyshire, J., Emery, S., Fatkenheuer, G., Gazzard, B., Grund, B., Hoy, J., Klingman, K., Losso, M., Markowitz, N., Neuhaus, J., Phillips, A., Rappoport, C., 2006. CD4+ count-guided interruption of antiretroviral treatment. N. Engl. J. Med. 355, 2283–2296.

Ermolieff, J., Lin, X., Tang, J., 1997. Kinetic properties of saquinavir-resistant mutants of human immunodeficiency virus type 1 protease and their implications in drug resistance in vivo. Biochemistry 36, 12364–12370.

Flexner, C., 1998. HIV-protease inhibitors. N. Engl. J. Med. 338, 1281–1292.

Flys, T.S., Mwatha, A., Guay, L.A., Nakabiito, C., Donnell, D., Musoke, P., Mmiro, F., Jackson, J.B., Eshleman, S.H., 2007. Detection of K103N in Ugandan women after repeated exposure to single dose nevirapine. Aids 21, 2077–2082.

Friend, J., Parkin, N., Liegler, T., Martin, J.N., Deeks, S.G., 2004. Isolated lopinavir resistance after virological rebound of a ritonavir/lopinavir-based regimen. Aids 18, 1965–1966.

Frost, S.D., Nijhuis, M., Schuurman, R., Boucher, C.A., Brown, A.J., 2000. Evolution of lamivudine resistance in human immunodeficiency virus type 1-infected individuals: the relative roles of drift and selection. J. Virol. 74, 6262–6268.

Gao, Q., Gu, Z., Parniak, M.A., Cameron, J., Cammack, N., Boucher, C., Wainberg, M.A., 1993. The same mutation that encodes low-level human immunodeficiency virus type 1 resistance to 2′,3′-dideoxyinosine and 2′,3′-dideoxycytidine confers high-level resistance to the (−) enantiomer of 2′,3′-dideoxy-3′-thiacytidine. Antimicrob. Agents Chemother. 37, 1390–1392.

Garcia-Lerma, J.G., MacInnes, H., Bennett, D., Reid, P., Nidtha, S., Weinstock, H., Kaplan, J.E., Heneine, W., 2003. A novel genetic pathway of human immunodeficiency virus type 1 resistance to stavudine mediated by the K65R mutation. J. Virol. 77, 5685–5693.

Grant, P., Wong, E.C., Rode, R., Shafer, R., De Luca, A., Nadler, J., Hawkins, T., Cohen, C., Harrington, R., Kempf, D., Zolopa, A., 2008. Virologic response to lopinavir-ritonavir-based antiretroviral regimens in a multicenter international clinical cohort: comparison of genotypic interpretation scores. Antimicrob. Agents Chemother. 52, 4050–4056.

Grant, R.M., Hecht, F.M., Warmerdam, M., Liu, L., Liegler, T., Petropoulos, C.J., Hellmann, N.S., Chesney, M., Busch, M.P., Kahn, J.O., 2002. Time trends in primary HIV-1 drug resistance among recently infected persons. JAMA 288, 181–188.

Greenberg, M.L., Cammack, N., 2004. Resistance to enfuvirtide, the first HIV fusion inhibitor. J. Antimicrob. Chemother. 54, 333–340.

Gu, Z., Arts, E.J., Parniak, M.A., Wainberg, M.A., 1995a. Mutated K65R recombinant reverse transcriptase of human immunodeficiency virus type 1 shows diminished chain termination in the presence of 2′,3′-dideoxycytidine 5′-triphosphate and other drugs. Proc. Natl. Acad. Sci. USA 92, 2760–2764.

Gu, Z., Fletcher, R.S., Arts, E.J., Wainberg, M.A., Parniak, M.A., 1994. The K65R mutant reverse transcriptase of HIV-1 cross-resistant to 2′, 3′-dideoxycytidine, 2′,3′-dideoxy-3′-thiacytidine, and 2′,3′-dideoxyinosine shows reduced sensitivity to specific dideoxynucleoside triphosphate inhibitors in vitro. J. Biol. Chem. 269, 28118–28122.

Gu, Z., Salomon, H., Cherrington, J.M., Mulato, A.S., Chen, M.S., Yarchoan, R., Foli, A., Sogocio, K.M., Wainberg, M.A., 1995b. K65R mutation of human immunodeficiency virus type 1 reverse transcriptase encodes cross-resistance to 9-(2-phosphonylmethoxyethyl)adenine. Antimicrob. Agents Chemother. 39, 1888–1891.

Gulick, R.M., Ribaudo, H.J., Shikuma, C.M., Lustgarten, S., Squires, K.E., Meyer 3rd, W.A., Acosta, E.P., Schackman, B.R., Pilcher, C.D., Murphy, R.L., Maher, W.E., Witt, M.D., Reichman, R.C., Snyder, S., Klingman, K.L., Kuritzkes, D.R., 2004. Triple-nucleoside regimens versus efavirenz-containing regimens for the initial treatment of HIV-1 infection. N. Engl. J. Med. 350, 1850–1861.

Gulnik, S.V., Suvorov, L.I., Liu, B., Yu, B., Anderson, B., Mitsuya, H., Erickson, J.W., 1995. Kinetic characterization and cross-resistance patterns of HIV-1 protease mutants selected under drug pressure. Biochemistry 34, 9282–9287.

Gupta, R., Lall, M., Sen, S., Kapila, K., Chopra, G., Paranjape, R., Tripathy, S., 2008. Profile of primary resistance in HIV-1 infected antiretroviral naive individuals from Western India. In: XVII International AIDS Conference, 3–8 August 2008, Mexico City (abstract LBPE1131).

Gupta, S., Fransen, S., Paxinos, E., Huang, W., Stawiski, E., Petropoulos, C., Parkin, N., 2006a. Infrequent occurrence of mutations in the C-terminal region of reverse transcriptase modulates susceptibility to RT inhibitors. 15th International HIV Drug Resistance Workshop, 13–17 June 2006, Sitges, Spain. Antivir. Ther. 11, S143 (abstract 127).

Gupta, S., Fransen, S., Paxinos, E., Huang, W., Stawiski, E., Petropoulos, C., Parkin, N., 2006b. Infrequent occurrence of mutations in the C-terminal region of reverse transcriptase modulates susceptibility to RT inhibitors. Antivir. Ther., 11.

Hamers, R., Wallis, C., Siwale, M., van Hasselt, R., Labib, M., Derdelinckx, I., van Vugt, M., Stevens, W., Schuurman, R., T, R.D.W., 2008. Implementation of the PharmAccess African studies to evaluate HIV drug resistance (PASER) program: preliminary baseline results of a patient cohort in Lusaka, Zambia. In: XVII International AIDS Conference, 3–8 August 2008, Mexico City (abstract MOPDA204).

Hammer, S.M., Eron Jr., J.J., Reiss, P., Schooley, R.T., Thompson, M.A., Walmsley, S., Cahn, P., Fischl, M.A., Gatell, J.M., Hirsch, M.S., Jacobsen, D.M., Montaner, J.S., Richman, D.D., Yeni, P.G., Volberding, P.A., 2008. Antiretroviral treatment of adult HIV infection: 2008 recommendations of the International AIDS Society-USA panel. JAMA 300, 555–570.

Harrigan, P.R., Hertogs, K., Verbiest, W., Pauwels, R., Larder, B., Kemp, S., Bloor, S., Yip, B., Hogg, R., Alexander, C., Montaner, J.S., 1999. Baseline HIV drug resistance profile predicts response to ritonavir-saquinavir protease inhibitor therapy in a community setting. Aids 13, 1863–1871.

Hatano, H., Hunt, P., Weidler, J., Coakley, E., Hoh, R., Liegler, T., Martin, J.N., Deeks, S.G., 2006. Rate of viral evolution and risk of losing future drug options in heavily pretreated, HIV-infected patients who continue to receive a stable, partially suppressive treatment regimen. Clin. Infect. Dis. 43, 1329–1336.

Havlir, D.V., Hellmann, N.S., Petropoulos, C.J., Whitcomb, J.M., Collier, A.C., Hirsch, M.S., Tebas, P., Sommadossi, J.P., Richman, D.D., 2000. Drug susceptibility in HIV infection after viral rebound in patients receiving indinavir-containing regimens. JAMA 283, 229–234.

Hazuda, D., Miller, M., Nguyen, B., Zhao, J., The P005 Study Team, Abstract 8 2007. Resistance to the HIV-integrase inhibitor raltegravir: analysis of protocol 005, a phase II study in patients with triple-class resistant HIV-1 infection. In: Program and Abstracts of the XVI International HIV Drug Resistance Workshop, June 12–16, St. Michael, Barbados.

Hicks, C.B., Cahn, P., Cooper, D.A., Walmsley, S.L., Katlama, C., Clotet, B., Lazzarin, A., Johnson, M.A., Neubacher, D., Mayers, D., Valdez, H., 2006. Durable efficacy of tipranavir-ritonavir in combination with an optimised background regimen of antiretroviral drugs for treatment-experienced HIV-1-infected patients at 48 weeks in the Randomized Evaluation of Strategic Intervention in multi-drug reSistant patients with Tipranavir (RESIST) studies: an analysis of combined data from two randomised open-label trials. Lancet 368, 466–475.

Hirsch, M.S., Gunthard, H.F., Schapiro, J.M., Brun-Vezinet, F., Clotet, B., Hammer, S.M., Johnson, V.A., Kuritzkes, D.R., Mellors, J.W., Pillay, D., Yeni, P.G., Jacobsen, D.M., Richman, D.D., 2008. Antiretroviral drug resistance testing in adult HIV-1 infection: 2008 recommendations of an International AIDS Society-USA panel. Clin. Infect. Dis. 47, 266–285.

Hogg, R.S., Bangsberg, D.R., Lima, V.D., Alexander, C., Bonner, S., Yip, B., Wood, E., Dong, W.W., Montaner, J.S., Harrigan, P.R., 2006. Emergence of drug resistance is associated with an increased risk of death among patients first starting HAART. PLoS Med., 3.

Hsiou, Y., Ding, J., Das, K., Clark Jr., A.D., Boyer, P.L., Lewi, P., Janssen, P.A., Kleim, J.P., Rosner, M., Hughes, S.H., Arnold, E., 2001. The Lys103Asn mutation of HIV-1 RT: a novel mechanism of drug resistance. J. Mol. Biol. 309, 437–445.

Huang, H., Chopra, R., Verdine, G.L., Harrison, S.C., 1998. Structure of a covalently trapped catalytic complex of HIV-1 reverse transcriptase: implications for drug resistance. Science 282, 1669–1675.

Ji, J., Loeb, L.A., 1994. Fidelity of HIV-1 reverse transcriptase copying a hypervariable region of the HIV-1 env gene. Virology 199, 323–330.

Johnson, J.A., Li, J.F., Morris, L., Martinson, N., Gray, G., McIntyre, J., Heneine, W., 2005. Emergence of drug-resistant HIV-1 after intrapartum administration of single-dose nevirapine is substantially underestimated. J. Infect. Dis. 192, 16–23.

Johnson, J.A., Li, J.F., Wei, X., Lipscomb, J., Irlbeck, D., Craig, C., Smith, A., Bennett, D.E., Monsour, M., Sandstrom, P., Lanier, E.R., Heneine, W., 2008. Minority HIV-1 drug resistance mutations are present in antiretroviral treatment-naive populations and associate with reduced treatment efficacy. PLoS Med. 5, e158.

Johnson, V.A., Brun-Vezinet, F., Clotet, B., Kuritzkes, D.R., Pillay, D., Schapiro, J.M., Richman, D.D., 2006. Update of the drug resistance mutations in HIV-1: Fall 2006. Top. HIV Med. 14, 125–130.

Kagan, R.M., Shenderovich, M.D., Heseltine, P.N., Ramnarayan, K., 2005. Structural analysis of an HIV-1 protease I47A mutant resistant to the protease inhibitor lopinavir. Protein Sci. 14, 1870–1878.

Kapoor, A., Jones, M., Shafer, R.W., Rhee, S.Y., Kazanjian, P., Delwart, E.L., 2004. Sequencing-based detection of low-frequency human immunodeficiency virus type 1 drug-resistant mutants by an RNA/DNA heteroduplex generator-tracking assay. J. Virol. 78, 7112–7123.

Katlama, C., Ingrand, D., Loveday, C., Clumeck, N., Mallolas, J., Staszewski, S., Johnson, M., Hill, A.M., Pearce, G., McDade, H., Lamivudine European HIV Working Group, 1996. Safety and efficacy of lamivudine-zidovudine combination therapy in antiretroviral-naive patients. A randomized controlled comparison with zidovudine monotherapy. JAMA 276, 118–125.

Kempf, D.J., Isaacson, J.D., King, M.S., Brun, S.C., Xu, Y., Real, K., Bernstein, B.M., Japour, A.J., Sun, E., Rode, R.A., 2001. Identification of genotypic changes in human immunodeficiency virus protease that correlate with reduced susceptibility to the protease inhibitor lopinavir among viral isolates from protease inhibitor-experienced patients. J. Virol. 75, 7462–7469.

King, M.S., Rode, R., Cohen-Codar, I., Calvez, V., Marcelin, A.G., Hanna, G.J., Kempf, D.J., 2007. Predictive genotypic algorithm for virologic response to lopinavir-ritonavir in protease inhibitor-experienced patients. Antimicrob. Agents Chemother. 51, 3067–3074.

Klabe, R.M., Bacheler, L.T., Ala, P.J., Erickson-Viitanen, S., Meek, J.L., 1998. Resistance to HIV protease inhibitors: a comparison of enzyme inhibition and antiviral potency. Biochemistry 37, 8735–8742.

Koot, M., Keet, I.P., Vos, A.H., de Goede, R.E., Roos, M.T., Coutinho, R.A., Miedema, F., Schellekens, P.T., Tersmette, M., 1993. Prognostic value of HIV-1 syncytium-inducing phenotype for rate of CD4+cell depletion and progression to AIDS. Ann. Intern. Med. 118, 681–688.

Kozal, M.J., Hullsiek, K.H., Macarthur, R.D., Berg-Wolf, M., Peng, G., Xiang, Y., Baxter, J.D., Uy, J., Telzak, E.E., Novak, R.M., 2007. The Incidence of HIV drug resistance and its impact on progression of HIV disease among antiretroviral-naive participants started on three different antiretroviral therapy strategies. HIV Clin. Trials 8, 357–370.

Kuritzkes, D.R., Lalama, C.M., Ribaudo, H.J., Marcial, M., Meyer 3rd, W.A., Shikuma, C., Johnson, V.A., Fiscus, S.A., D'Aquila, R.T., Schackman, B.R., Acosta, E.P., Gulick, R.M., 2008. Preexisting resistance to nonnucleoside reverse-transcriptase inhibitors predicts virologic failure of an efavirenz-based regimen in treatment-naive HIV-1-infected subjects. J. Infect. Dis. 197, 867–870.

Kuritzkes, D.R., Quinn, J.B., Benoit, S.L., Shugarts, D.L., Griffin, A., Bakhtiari, M., Poticha, D., Eron, J.J., Fallon, M.A., Rubin, M., 1996. Drug resistance and virologic response in NUCA 3001, a randomized trial of lamivudine (3TC) versus zidovudine (ZDV) versus ZDV plus 3TC in previously untreated patients. Aids 10, 975–981.

Lam, E., Parkin, N.T., 2003. Amprenavir resistance imparted by the I50V mutation in HIV-1 protease can be suppressed by the N88S mutation. Clin. Infect. Dis. 37, 1273–1274.

Larder, B.A., Darby, G., Richman, D.D., 1989. HIV with reduced sensitivity to zidovudine (AZT) isolated during prolonged therapy. Science 243, 1731–1734.

Larder, B.A., Kellam, P., Kemp, S.D., 1991. Zidovudine resistance predicted by direct detection of mutations in DNA from HIV-infected lymphocytes. Aids 5, 137–144.

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

Lastere, S., Dalban, C., Collin, G., Descamps, D., Girard, P.M., Clavel, F., Costagliola, D., Brun-Vezinet, F., 2004. Impact of insertions in the HIV-1 p6 PTAPP region on the virological response to amprenavir. Antivir. Ther. 9, 221–227.

Lawrence, J., Mayers, D.L., Hullsiek, K.H., Collins, G., Abrams, D.I., Reisler, R.B., Crane, L.R., Schmetter, B.S., Dionne, T.J., Saldanha, J.M., Jones, M.C., Baxter, J.D., 2003. Structured treatment interruption in patients with multidrug-resistant human immunodeficiency virus. N. Engl. J. Med. 349, 837–846.

Le, T., Chiarella, J., Simen, B.B., Hanczaruk, B., Egholm, M., Landry, M.L., Dieckhaus, K., Rosen, M.I., Kozal, M.J., 2009. Low-abundance HIV drug-resistant viral variants in treatment-experienced persons correlate with historical antiretroviral use. PLoS ONE 4, e6079.

Lecossier, D., Shulman, N.S., Morand-Joubert, L., Shafer, R.W., Joly, V., Zolopa, A.R., Clavel, F., Hance, A.J., 2005. Detection of minority populations of HIV-1 expressing the K103N resistance mutation in patients failing nevirapine. J. Acquir. Immune Defic. Syndr. 38, 37–42.

Leigh Brown, A.J., Frost, S.D., Mathews, W.C., Dawson, K., Hellmann, N.S., Daar, E.S., Richman, D.D., Little, S.J., 2003. Transmission fitness of drug-resistant human immunodeficiency virus and the prevalence of resistance in the antiretroviral-treated population. J. Infect. Dis. 187, 683–686.

Little, S.J., 2000. Transmission and prevalence of HIV resistance among treatment-naive subjects. Antivir. Ther. 5, 33–40.

Little, S.J., Daar, E.S., D'Aquila, R.T., Keiser, P.H., Connick, E., Whitcomb, J.M., Hellmann, N.S., Petropoulos, C.J., Sutton, L., Pitt, J.A., Rosenberg, E.S., Koup, R.A., Walker, B.D., Richman, D.D., 1999. Reduced antiretroviral drug susceptibility among patients with primary HIV infection. JAMA 282, 1142–1149.

Little, S.J., Frost, S.D.W., Wong, J.K., Smith, D.M., Pond, S.L.K., Ignacio, C.C., Parkin, N.T., Petropoulos, C.J., Richman, D.D., 2008. Persistence of transmitted drug resistance among subjects with primary human immunodeficiency virus infection. J. Virol. 82, 5510–5518.

Little, S.J., Holte, S., Routy, J.P., Daar, E.S., Markowitz, M., Collier, A.C., Koup, R.A., Mellors, J.W., Connick, E., Conway, B., Kilby, M., Wang, L., Whitcomb, J.M., Hellmann, N.S., Richman, D.D., 2002. Antiretroviral-drug resistance among patients recently infected with HIV. N. Engl. J. Med. 347, 385–394.

Lohse, N., Jorgensen, L.B., Kronborg, G., Moller, A., Kvinesdal, B., Sorensen, H.T., Obel, N., Gerstoft, J., 2007. Genotypic drug resistance and long-term mortality in patients with triple-class antiretroviral drug failure. Antivir. Ther. 12, 909–917.

Loubser, S., Balfe, P., Sherman, G., Hammer, S., Kuhn, L., Morris, L., 2006. Decay of K103N mutants in cellular DNA and plasma RNA after single-dose nevirapine to reduce mother-to-child HIV transmission. Aids 20, 995–1002.

Maguire, M.F., Guinea, R., Griffin, P., Macmanus, S., Elston, R.C., Wolfram, J., Richards, N., Hanlon, M.H., Porter, D.J., Wrin, T., Parkin, N., Tisdale, M., Furfine, E., Petropoulos, C., Snowden, B.W., Kleim, J.P., 2002. Changes in human immunodeficiency virus type 1 Gag at positions L449 and P453 are linked to I50V protease mutants

in vivo and cause reduction of sensitivity to amprenavir and improved viral fitness in vitro. J. Virol. 76, 7398–7406.
Marcelin, A.G., Affolabi, D., Lamotte, C., Mohand, H.A., Delaugerre, C., Wirden, M., Voujon, D., Bossi, P., Ktorza, N., Bricaire, F., Costagliola, D., Katlama, C., Peytavin, G., Calvez, V., 2004. Resistance profiles observed in virological failures after 24 weeks of amprenavir/ritonavir containing regimen in protease inhibitor experienced patients. J. Med. Virol. 74, 16–20.
Marcelin, A.G., Lamotte, C., Delaugerre, C., Ktorza, N., Ait Mohand, H., Cacace, R., Bonmarchand, M., Wirden, M., Simon, A., Bossi, P., Bricaire, F., Costagliola, D., Katlama, C., Peytavin, G., Calvez, V., 2003. Genotypic inhibitory quotient as predictor of virological response to ritonavir-amprenavir in human immunodeficiency virus type 1 protease inhibitor-experienced patients. Antimicrob. Agents Chemother. 47, 594–600.
Marconi, V.C., Sunpath, H., Lu, Z., Gordon, M., Koranteng-Apeagyei, K., Hampton, J., Carpenter, S., Giddy, J., Ross, D., Holst, H., Losina, E., Walker, B.D., Kuritzkes, D.R., 2008. Prevalence of HIV-1 drug resistance after failure of a first highly active antiretroviral therapy regimen in KwaZulu Natal, South Africa. Clin. Infect. Dis. 46, 1589–1597.
Martinez-Picado, J., Morales-Lopetegi, K., Wrin, T., Prado, J.G., Frost, S.D., Petropoulos, C.J., Clotet, B., Ruiz, L., 2002. Selection of drug-resistant HIV-1 mutants in response to repeated structured treatment interruptions. Aids 16, 895–899.
Martinez-Picado, J., Wrin, T., Frost, S.D., Clotet, B., Ruiz, L., Brown, A.J., Petropoulos, C.J., Parkin, N.T., 2005. Phenotypic hypersusceptibility to multiple protease inhibitors and low replicative capacity in patients who are chronically infected with human immunodeficiency virus type 1. J. Virol. 79, 5907–5913.
Masquelier, B., Breilh, D., Neau, D., Lawson-Ayayi, S., Lavignolle, V., Ragnaud, J.M., Dupon, M., Morlat, P., Dabis, F., Fleury, H., 2002. Human immunodeficiency virus type 1 genotypic and pharmacokinetic determinants of the virological response to lopinavir-ritonavir-containing therapy in protease inhibitor-experienced patients. Antimicrob. Agents Chemother. 46, 2926–2932.
McColl, D., Fransen, S., Gupta, S., Parkin, N., Margot, N., Chuck, S., Cheng, A., Miller, M., Resistance, 2007. Cross-resistance to first generation integrase inhibitors: insights from a phase II study of Elvitegravir (GS-9137). Antivir. Ther. 12, S11 (abstract no. 9).
Metzner, K.J., Bonhoeffer, S., Fischer, M., Karanicolas, R., Allers, K., Joos, B., Weber, R., Hirschel, B., Kostrikis, L.G., Gunthard, H.F., 2003. Emergence of minor populations of human immunodeficiency virus type 1 carrying the M184V and L90M mutations in subjects undergoing structured treatment interruptions. J. Infect. Dis. 188, 1433–1443.
Metzner, K.J., Giulieri, S.G., Knoepfel, S.A., Rauch, P., Burgisser, P., Yerly, S., Gunthard, H.F., Cavassini, M., 2009. Minority quasispecies of drug-resistant HIV-1 that lead to early therapy failure in treatment-naive and -adherent patients. Clin. Infect. Dis. 48, 239–247.
Metzner, K.J., Rauch, P., Walter, H., Boesecke, C., Zollner, B., Jessen, H., Schewe, K., Fenske, S., Gellermann, H., Stellbrink, H.J., 2005. Detection of minor populations of drug-resistant HIV-1 in acute seroconverters. Aids 19, 1819–1825.
Miller, M.D., Margot, N.A., Lamy, P.D., Fuller, M.D., Anton, K.E., Mulato, A.S., Cherrington, J.M., 2001. Adefovir and tenofovir susceptibilities of HIV-1 after 24 to 48 weeks of adefovir dipivoxil therapy: genotypic and phenotypic analyses of study GS-96-408. J. Acquir. Immune Defic. Syndr. 27, 450–458.
Miller, V., Ait-Khaled, M., Stone, C., Griffin, P., Mesogiti, D., Cutrell, A., Harrigan, R., Staszewski, S., Katlama, C., Pearce, G., Tisdale, M., 2000. HIV-1 reverse transcriptase (RT) genotype and susceptibility to RT inhibitors during abacavir monotherapy and combination therapy. Aids 14, 163–171.
Miller, V., Larder, B.A., 2001. Mutational patterns in the HIV genome and cross-resistance following nucleoside and nucleotide analogue drug exposure. Antivir. Ther. 6 (Suppl. 3), 25–44.
Miller, V., Phillips, A., Rottmann, C., Staszewski, S., Pauwels, R., Hertogs, K., de Bethune, M.P., Kemp, S.D., Bloor, S., Harrigan, P.R., Larder, B.A., 1998. Dual resistance to zidovudine and lamivudine in patients treated with zidovudine-lamivudine combination therapy: association with therapy failure. J. Infect. Dis. 177, 1521–1532.
Mo, H., King, M.S., King, K., Molla, A., Brun, S., Kempf, D.J., 2005. Selection of resistance in protease inhibitor-experienced, human immunodeficiency virus type 1-infected subjects failing lopinavir- and ritonavir-based therapy: mutation patterns and baseline correlates. J. Virol. 79, 3329–3338.
Molla, A., Korneyeva, M., Gao, Q., Vasavanonda, S., Schipper, P.J., Mo, H.M., Markowitz, M., Chernyavskiy, T., Niu, P., Lyons, N., Hsu, A., Granneman, G.R., Ho, D.D., Boucher, C.A., Leonard, J.M., Norbeck, D.W., Kempf, D.J., 1996. Ordered accumulation of mutations in HIV protease confers resistance to ritonavir. Nat. Med. 2, 760–766.
Monno, L., Saracino, A., Scudeller, L., Pastore, G., Bonora, S., Cargnel, A., Carosi, G., Angarano, G., 2003. HIV-1 phenotypic susceptibility to lopinavir (LPV) and genotypic analysis in LPV/r-naive subjects with prior protease inhibitor experience. J. Acquir. Immune Defic. Syndr. 33, 439–447.
Moyle, G.J., 2004. The K65R mutation: selection, frequency, and possible consequences. AIDS Read. 14, 595–597, 601–603.
Nachega, J.B., Hislop, M., Dowdy, D.W., Chaisson, R.E., Regensberg, L., Maartens, G., 2007. Adherence to nonnucleoside reverse transcriptase inhibitor-based HIV therapy and virologic outcomes. Ann. Intern. Med. 146, 564–573.
Nachega, J.B., Lehman, D.A., Hlatshwayo, D., Mothopeng, R., Chaisson, R.E., Karstaedt, A.S., 2005. HIV/AIDS and antiretroviral treatment knowledge, attitudes, beliefs, and practices in HIV-infected adults in Soweto, South Africa. J. Acquir. Immune Defic. Syndr. 38, 196–201.
Naeger, L.K., Struble, K.A., 2007. Food and Drug Administration analysis of tipranavir clinical resistance in HIV-1-infected treatment-experienced patients. Aids 21, 179–185.
Naugler, W.E., Yong, F.H., Carey, V.J., Dragavon, J.A., Coombs, R.W., Frenkel, L.M., 2002. T69D/N pol mutation, human immunodeficiency virus type 1 RNA levels, and syncytium-inducing phenotype are associated with CD4 cell depletion during didanosine therapy. J. Infect. Dis. 185, 448–455.
Nijhuis, M., van Maarseveen, N.M., Lastere, S., Schipper, P., Coakley, E., Glass, B., Rovenska, M., de Jong, D., Chappey, C., Goedegebuure, I.W., Heilek-Snyder, G., Dulude, D., Cammack, N., Brakier-Gingras, L., Konvalinka, J., Parkin, N., Krausslich, H.G., Brun-Vezinet, F., Boucher, C.A., 2007. A novel substrate-based HIV-1 protease inhibitor drug resistance mechanism. PLoS Med 4, e36.
Nikolenko, G.N., Svarovskaia, E.S., Delviks, K.A., Pathak, V.K., 2004. Antiretroviral drug resistance mutations in human immunodeficiency virus type 1 reverse transcriptase increase template-switching frequency. J. Virol. 78, 8761–8770.
Nyombi, B.M., Holm-Hansen, C., Kristiansen, K.I., Bjune, G., Muller, F., 2008. Prevalence of reverse transcriptase and protease mutations associated with antiretroviral drug resistance among drug-naive HIV-1 infected pregnant women in Kagera and Kilimanjaro regions, Tanzania. AIDS Res. Ther. 5, 13.
Palmer, S., Boltz, V., Martinson, N., Maldarelli, F., Gray, G., McIntyre, J., Mellors, J., Morris, L., Coffin, J., 2006. Persistence of nevirapine-resistant HIV-1 in women after single-dose nevirapine therapy for prevention of maternal-to-fetal HIV-1 transmission. Proc. Natl. Acad. Sci. USA 103, 7094–7099.
Paredes, R., Cheng, I., Kuritzkes, D.R., Tuomala, R.E., 2007a. High prevalence of primary lamivudine and nelfinavir resistance in HIV-1-infected pregnant women in the United States, 1998–2004. Aids 21, 2103–2106.
Paredes, R., Lalama, C., Ribaudo, H., Schackman, B., Shikuma, C., Meyer, W., Giguel, F., Squires, K., Gulick, R., Kuritzkes, D.R., Paredes, R., Marconi, V.C., Campbell, T.B., Kuritzkes, D.R., 2007b. Systematic evaluation of allele-specific real-time PCR for the detection of minor HIV-1 variants with pol and env resistance mutations. J. Virol. Methods.
Paredes, R., Sagar, M., Marconi, V.C., Hoh, R., Martin, J.N., Parkin, N.T., Petropoulos, C.J., Deeks, S.G., Kuritzkes, D.R., 2009. In vivo fitness cost of the M184V mutation in multidrug-resistant human immunodeficiency virus type 1 in the absence of lamivudine. J. Virol. 83, 2038–2043.
Parkin, N.T., Chappey, C., Petropoulos, C.J., 2003. Improving lopinavir genotype algorithm through phenotype correlations: novel mutation patterns and amprenavir cross-resistance. Aids 17, 955–961.
Peeters, M., Nijs, S., Vingerhoets, J., Tambuyzer, L., Woodfall, B., de Bethune, M.P., Picchio, G., 2008. Determination of phenotypic clinical cut-offs for etravirine: pooled week 24 results of the DUET-1 and DUET-2 trials. XVII HIV Drug Resistance Workshop, June 10–14, 2008, Sitges, Spain. Antivir. Ther. 13 (Suppl. 3), A134 (abstract no. 121).
Pellegrin, I., Wittkop, L., Joubert, L.M., Neau, D., Bollens, D., Bonarek, M., Girard, P.M., Fleury, H., Winters, B., Saux, M.C., Pellegrin, J.L., Thiebaut, R., Breilh, D., 2008. Virological response to darunavir/ritonavir-based regimens in antiretroviral-experienced patients (PREDIZISTA study). Antivir. Ther. 13, 271–279.
Peuchant, O., Thiebaut, R., Capdepont, S., Lavignolle-Aurillac, V., Neau, D., Morlat, P., Dabis, F., Fleury, H., Masquelier, B., 2008. Transmission of HIV-1 minority-resistant variants and response to first-line antiretroviral therapy. Aids 22, 1417–1423.
Picchio, G., Vingerhoets, J., Parkin, N., Azijn, H., de Bethune, M.P., 2008. Nucleoside-associated mutations cause hypersusceptibility to etravirine (ETR). XVII HIV Drug Resistance Workshop, June 10–14, 2008, Sitges, Spain. Antivir. Ther. 13 (Suppl. 3), A134 (abstract no. 23).
Pillay, D., Bhaskaran, K., Jurriaans, S., Prins, M., Masquelier, B., Dabis, F., Gifford, R., Nielsen, C., Pedersen, C., Balotta, C., Rezza, G., Ortiz, M., de Mendoza, C., Kucherer, C., Poggensee, G., Gill, J., Porter, K., 2006. The impact of transmitted drug resistance on the natural history of HIV infection and response to first-line therapy. Aids 20, 21–28.
Prado, J.G., Wrin, T., Beauchaine, J., Ruiz, L., Petropoulos, C.J., Frost, S.D., Clotet, B., D'Aquila, R.T., Martinez-Picado, J., 2002. Amprenavir-resistant HIV-1 exhibits lopinavir cross-resistance and reduced replication capacity. Aids 16, 1009–1017.
Quinones-Mateu, M.E., Arts, E.J., 2002. Fitness of drug resistant HIV-1: methodology and clinical implications. Drug Resist. Updat. 5, 224–233.
Ray, A.S., Basavapathruni, A., Anderson, K.S., 2002. Mechanistic studies to understand the progressive development of resistance in human immunodeficiency virus type 1 reverse transcriptase to abacavir. J. Biol. Chem. 277, 40479–40490.
Ren, J., Nichols, C., Bird, L., Chamberlain, P., Weaver, K., Short, S., Stuart, D.I., Stammers, D.K., 2001. Structural mechanisms of drug resistance for mutations at codons 181 and 188 in HIV-1 reverse transcriptase and the improved resilience of second generation non-nucleoside inhibitors. J. Mol. Biol. 312, 795–805.
Resch, W., Ziermann, R., Parkin, N., Gamarnik, A., Swanstrom, R., 2002. Nelfinavir-resistant, amprenavir-hypersusceptible strains of human immunodeficiency virus type 1 carrying an N88S mutation in protease have reduced infectivity, reduced replication capacity, and reduced fitness and process the Gag polyprotein precursor aberrantly. J. Virol. 76, 8659–8666.
Richman, D.D., 1990. Susceptibility to nucleoside analogues of zidovudine-resistant isolates of human immunodeficiency virus. Am. J. Med. 88, 8S–10S.
Richman, D.D., Havlir, D., Corbeil, J., Looney, D., Ignacio, C., Spector, S.A., Sullivan, J., Cheeseman, S., Barringer, K., Pauletti, D., et al., 1994. Nevirapine resistance mutations of human immunodeficiency virus type 1 selected during therapy. J. Virol. 68, 1660–1666.
Richman, D.D., Margolis, D.M., Delaney, M., Greene, W.C., Hazuda, D., Pomerantz, R.J., 2009. The challenge of finding a cure for HIV infection. Science 323, 1304–1307.

Richman, D.D., Morton, S.C., Wrin, T., Hellmann, N., Berry, S., Shapiro, M.F., Bozzette, S.A., 2004. The prevalence of antiretroviral drug resistance in the United States. Aids 18, 1393–1401.

Riddler, S.A., Haubrich, R., DiRienzo, A.G., Peeples, L., Powderly, W.G., Klingman, K.L., Garren, K.W., George, T., Rooney, J.F., Brizz, B., Lalloo, U.G., Murphy, R.L., Swindells, S., Havlir, D., Mellors, J.W., 2008. Class-sparing regimens for initial treatment of HIV-1 infection. N. Engl. J. Med. 358, 2095–2106.

Rimsky, L.T., Shugars, D.C., Matthews, T.J., 1998. Determinants of human immunodeficiency virus type 1 resistance to gp41-derived inhibitory peptides. J. Virol. 72, 986–993.

Rodrigues, R., Knoll, R., Nunes, C., Ferreira, J., Thomaz, M., Batista, J., Scherer, L., Brigido, L., 2008. Low primary HIV drug resistance mutations in South Brazil. In: XVII International AIDS Conference, 3–8 August 2008, Mexico City (abstract TUPE0027).

Roge, B.T., Katzenstein, T.L., Obel, N., Nielsen, H., Kirk, O., Pedersen, C., Mathiesen, L., Lundgren, J., Gerstoft, J., 2003. K65R with and without S68: a new resistance profile in vivo detected in most patients failing abacavir, didanosine and stavudine. Antivir. Ther. 8, 173–182.

Roquebert, B., Malet, I., Wirden, M., Tubiana, R., Valantin, M.A., Simon, A., Katlama, C., Peytavin, G., Calvez, V., Marcelin, A.G., 2006. Role of HIV-1 minority populations on resistance mutational pattern evolution and susceptibility to protease inhibitors. Aids 20, 287–289.

Ross, L., Dix, L., Wine, B., Vavro, C., Horton, J., Pappa, K., 2008. Changes in regional prevalence, clade and epidemiology of HIV-1 drug resistance mutations and clade among antiviral therapy-naive patients in the United States (US) from 2000 to 2007. In: XVII International HIV Drug Resistance Workshop, June 10–14, 2008, Sitges, Spain (abstract 146).

Sax, P.E., Islam, R., Walensky, R.P., Losina, E., Weinstein, M.C., Goldie, S.J., Sadownik, S.N., Freedberg, K.A., 2005. Should resistance testing be performed for treatment-naive HIV-infected patients? A cost-effectiveness analysis. Clin. Infect. Dis. 41, 1316–1323.

Schackman, B.R., Ribaudo, H.J., Krambrink, A., Hughes, V., Kuritzkes, D.R., Gulick, R.M., 2007. Racial differences in virologic failure associated with adherence and quality of life on efavirenz-containing regimens for initial HIV therapy: results of ACTG A5095. J. Acquir. Immune Defic. Syndr. 46, 547–554.

Schapiro, J.M., Winters, M.A., Lawrence, J., Merigan, T.C., 1999. Clinical cross-resistance between the HIV-1 protease inhibitors saquinavir and indinavir and correlations with genotypic mutations. Aids 13, 359–365.

Schinazi, R.F., Lloyd Jr., R.M., Nguyen, M.H., Cannon, D.L., McMillan, A., Ilksoy, N., Chu, C.K., Liotta, D.C., Bazmi, H.Z., Mellors, J.W., 1993. Characterization of human immunodeficiency viruses resistant to oxathiolane-cytosine nucleosides. Antimicrob. Agents Chemother. 37, 875–881.

Schuurman, R., Nijhuis, M., van Leeuwen, R., Schipper, P., de Jong, D., Collis, P., Danner, S.A., Mulder, J., Loveday, C., Christopherson, C., et al., 1995. Rapid changes in human immunodeficiency virus type 1 RNA load and appearance of drug-resistant virus populations in persons treated with lamivudine (3TC). J. Infect. Dis. 171, 1411–1419.

Shafer, R.W., Winters, M.A., Palmer, S., Merigan, T.C., 1998. Multiple concurrent reverse transcriptase and protease mutations and multidrug resistance of HIV-1 isolates from heavily treated patients. Ann. Intern. Med. 128, 906–911.

Shah, F.S., Curr, K.A., Hamburgh, M.E., Parniak, M., Mitsuya, H., Arnez, J.G., Prasad, V.R., 2000. Differential influence of nucleoside analog-resistance mutations K65R and L74V on the overall mutation rate and error specificity of human immunodeficiency virus type 1 reverse transcriptase. J. Biol. Chem. 275, 27037–27044.

Shao, W., Boltz, V.F., Kearney, M., Maldarelli, F., Mellors, J.M., Stewart, C., Levitsky, A., Volfovsky, N., Stephens, R.M., Coffin, J.M., 2009. Characterization of HIV-1 sequence artifacts introduced by bulk PCR and detected by 454 sequencing. XVIII International HIV Drug Resistance Workshop: Basic Principles & Clinical Implications, Fort Myers, FL, USA, 9–13 June 2009. Antivir. Ther. 14 (Suppl. 1), A123 (Abstract 104).

Sharma, P.L., Crumpacker, C.S., 1999. Decreased processivity of human immunodeficiency virus type 1 reverse transcriptase (RT) containing didanosine-selected mutation Leu74Val: a comparative analysis of RT variants Leu74Val and lamivudine-selected Met184Val. J. Virol. 73, 8448–8456.

Sherer, R., 2007. Darunavir in the treatment of HIV-1 infection: a viewpoint by Renslow Sherer. Drugs 67, 2802–2803.

Shet, A., Berry, L., Mohri, H., Mehandru, S., Chung, C., Kim, A., Jean-Pierre, P., Hogan, C., Simon, V., Boden, D., Markowitz, M., 2006. Tracking the prevalence of transmitted antiretroviral drug-resistant HIV-1: a decade of experience. J. Acquir. Immune Defic. Syndr. 41, 439–446.

Simen, B.B., Simons, J.F., Hullsiek, K.H., Novak, R.M., Macarthur, R.D., Baxter, J.D., Huang, C., Lubeski, C., Turenchalk, G.S., Braverman, M.S., Desany, B., Rothberg, J.M., Egholm, M., Kozal, M.J., 2009. Low-abundance drug-resistant viral variants in chronically HIV-infected, antiretroviral treatment-naive patients significantly impact treatment outcomes. J. Infect. Dis. 199, 693–701.

Sirivichayakul, S., Ruxrungtham, K., Ungsedhapand, C., Techasathit, W., Ubolyam, S., Chuenyam, T., Emery, S., Cooper, D., Lange, J., Phanuphak, P., 2003. Nucleoside analogue mutations and Q151M in HIV-1 subtype A/E infection treated with nucleoside reverse transcriptase inhibitors. Aids 17, 1889–1896.

St Clair, M.H., Martin, J.L., Tudor-Williams, G., Bach, M.C., Vavro, C.L., King, D.M., Kellam, P., Kemp, S.D., Larder, B.A., 1991. Resistance to ddI and sensitivity to AZT induced by a mutation in HIV-1 reverse transcriptase. Science 253, 1557–1559.

Sungkanuparph, S., Manosuthi, W., Kiertiburanakul, S., Piyavong, B., Chumpathat, N., Chantratita, W., 2007. Options for a second-line antiretroviral regimen for HIV type 1-infected patients whose initial regimen of a fixed-dose combination of stavudine, lamivudine, and nevirapine fails. Clin. Infect. Dis. 44, 447–452.

Svarovskaia, E.S., Margot, N.A., Bae, A.S., Waters, J.M., Goodman, D., Zhong, L., Borroto-Esoda, K., Miller, M.D., 2007. Low-level K65R mutation in HIV-1 reverse transcriptase of treatment-experienced patients exposed to abacavir or didanosine. J. Acquir. Immune Defic. Syndr. 46, 174–180.

Tisdale, M., Kemp, S.D., Parry, N.R., Larder, B.A., 1993. Rapid in vitro selection of human immunodeficiency virus type 1 resistant to 3′-thiacytidine inhibitors due to a mutation in the YMDD region of reverse transcriptase. Proc. Natl. Acad. Sci. USA 90, 5653–5656.

Tisdale, M., Myers, R.E., Maschera, B., Parry, N.R., Oliver, N.M., Blair, E.D., 1995. Cross-resistance analysis of human immunodeficiency virus type 1 variants individually selected for resistance to five different protease inhibitors. Antimicrob. Agents Chemother. 39, 1704–1710.

Tisdale, M., Schulze, T., Larder, B.A., Moelling, K., 1991. Mutations within the RNase H domain of human immunodeficiency virus type 1 reverse transcriptase abolish virus infectivity. J. Gen. Virol. 72 (Pt 1), 59–66.

Truong, H.M., Grant, R.M., McFarland, W., Kellogg, T., Kent, C., Louie, B., Wong, E., Klausner, J.D., 2006. Routine surveillance for the detection of acute and recent HIV infections and transmission of antiretroviral resistance. Aids 20, 2193–2197.

Tsibris, A.M., Korber, B., Arnaout, R., Russ, C., Lo, C.C., Leitner, T., Gaschen, B., Theiler, J., Paredes, R., Su, Z., Hughes, M.D., Gulick, R.M., Greaves, W., Coakley, E., Flexner, C., Nusbaum, C., Kuritzkes, D.R., 2009. Quantitative deep sequencing reveals dynamic HIV-1 escape and large population shifts during CCR5 antagonist therapy in vivo. PLoS ONE 4, e5683.

Tural, C., Ruiz, L., Holtzer, C., Schapiro, J., Viciana, P., Gonzalez, J., Domingo, P., Boucher, C., Rey-Joly, C., Clotet, B., 2002. Clinical utility of HIV-1 genotyping and expert advice: the Havana trial. Aids 16, 209–218.

van Marck, H., Dierynck, I., Kraus, G., Hallenberger, S., Pattery, T., Muyldermans, G., Van Vlijmen, H., Hertogs, K., de Bethune, M.P., 2007. Unravelling the complex resistance pathways of darunavir using Bioinformatics Resistance Determination (BIRD). 16th International HIV Drug Resistance Workshop, 12–16 June 2007, Barbados, West Indies, USA. Antivir. Ther. 12, S141 (abstract no. 128).

Vandamme, A.M., Sonnerborg, A., Ait-Khaled, M., Albert, J., Asjo, B., Bacheler, L., Banhegyi, D., Boucher, C., Brun-Vezinet, F., Camacho, R., Clevenbergh, P., Clumeck, N., Dedes, N., De Luca, A., Doerr, H.W., Faudon, J.L., Gatti, G., Gerstoft, J., Hall, W.W., Hatzakis, A., Hellmann, N., Horban, A., Lundgren, J.D., Kempf, D., Miller, M., Miller, V., Myers, T.W., Nielsen, C., Opravil, M., Palmisano, L., Perno, C.F., Phillips, A., Pillay, D., Pumarola, T., Ruiz, L., Salminen, M., Schapiro, J., Schmidt, B., Schmit, J.C., Schuurman, R., Shulse, E., Soriano, V., Staszewski, S., Vella, S., Youle, M., Ziermann, R., Perrin, L., 2004. Updated European recommendations for the clinical use of HIV drug resistance testing. Antivir. Ther. 9, 829–848.

Vingerhoets, J., Peeters, M., Azijn, H., Tambuyzer, L., Hoogstoel, A., Nijs, S., de Béthune, M.P., Picchio, G., 2008. An update on the list of NNRTI mutations associated with decreased virological response to etravirine: multivariate analysis on the pooled DUET-1 and DUET-2 clinical trial data. XVII HIV Drug Resistance Workshop, June 10–14, 2008, Sitges, Spain. Antivir. Ther. 13 (Suppl. 3), A134 (abstract 24).

vonWyl, V., Yerly, S., Boni, J., Burgisser, P., Klimkait, T., Bonhoeffer, S., Ledergerber, B., Gunthard, H., (SHCS), a.t.S.H.C.S., 2008. The proportion of individuals without further treatment options has stabilized at low levels in the Swiss HIV Cohort Study. In: 5th Conference on Retroviruses and Opportunistic Infections, (Vol. Abstract 896).

Vray, M., Meynard, J.L., Dalban, C., Morand-Joubert, L., Clavel, F., Brun-Vezinet, F., Peytavin, G., Costagliola, D., Girard, P.M., 2003. Predictors of the virological response to a change in the antiretroviral treatment regimen in HIV-1-infected patients enrolled in a randomized trial comparing genotyping, phenotyping and standard of care (Narval trial, ANRS 088). Antivir. Ther. 8, 427–434.

Wainberg, M.A., 2004. The impact of the M184V substitution on drug resistance and viral fitness. Expert Rev Anti-Infect. Ther. 2, 147–151.

Wainberg, M.A., Drosopoulos, W.C., Salomon, H., Hsu, M., Borkow, G., Parniak, M., Gu, Z., Song, Q., Manne, J., Islam, S., Castriota, G., Prasad, V.R., 1996. Enhanced fidelity of 3TC-selected mutant HIV-1 reverse transcriptase. Science 271, 1282–1285.

Wainberg, M.A., Salomon, H., Gu, Z., Montaner, J.S., Cooley, T.P., McCaffrey, R., Ruedy, J., Hirst, H.M., Cammack, N., Cameron, J., et al., 1995. Development of HIV-1 resistance to (−)2′-deoxy-3′-thiacytidine in patients with AIDS or advanced AIDS-related complex. AIDS 9, 351–357.

Walter, H., Low, P., Harrer, T., Schmitt, M., Schwingel, E., Tschochner, M., Helm, M., Korn, K., Uberla, K., Schmidt, B., 2002. No evidence for persistence of multidrug-resistant viral strains after a 7-month treatment interruption in an HIV-1-infected individual. J. Acquir. Immune Defic. Syndr. 31, 137–146.

Wei, X., Decker, J.M., Liu, H., Zhang, Z., Arani, R.B., Kilby, J.M., Saag, M.S., Wu, X., Shaw, G.M., Kappes, J.C., 2002. Emergence of resistant human immunodeficiency virus type 1 in patients receiving fusion inhibitor (T-20) monotherapy. Antimicrob. Agents Chemother. 46, 1896–1905.

Wensing, A.M., van de Vijver, D.A., Angarano, G., Asjö, B., Balotta, C., Boeri, E., Camacho, R., Chaix, M.L., Costagliola, D., De Luca, A., Derdelinckx, I., Grossman, Z., Hamouda, O., Hatzakis, A., Hemmer, R., Hoepelman, A., Horban, A., Korn, K., Kücherer, C., Leitner, T., Loveday, C., MacRae, E., Maljkovic, I., de Mendoza, C., Meyer, L., Nielsen, C., Op de Coul, E.L., Ormaasen, V., Paraskevis, D., Perrin, L., Puchhammer-Stockl, E., Ruiz, L., Salminen, M., Schmit, J.C., Schneider, F., Schuurman, R., Soriano, V., Stanczak, G., Stanojevic, M., Vandamme, A.M., Van Laethem, K., Violin, M., Wilbe, K., Yerly, S., Zazzi, M., Boucher, C.A., 2005. Prevalence of drug-resistant HIV-1 variants in untreated individuals in Europe: implications for clinical management. J. Infect. Dis. 192, 958–966.

Westby, M., Lewis, M., Whitcomb, J., Youle, M., Pozniak, A.L., James, I.T., Jenkins, T.M., Perros, M., van der Ryst, E., 2006. Emergence of CXCR4-using human immunodeficiency virus type 1 (HIV-1) variants in a minority of HIV-1-infected patients

following treatment with the CCR5 antagonist maraviroc is from a pretreatment CXCR4-using virus reservoir. J. Virol. 80, 4909–4920.
Winston, A., Stebbing, J., 2004. The K65R mutation in HIV-1 reverse transcriptase. J. HIV Ther. 9, 25–27.
Winters, B., Montaner, J., Harrigan, P.R., Gazzard, B., Pozniak, A., Miller, M.D., Emery, S., van Leth, F., Robinson, P., Baxter, J.D., Perez-Elias, M., Castor, D., Hammer, S., Rinehart, A., Vermeiren, H., Van Craenenbroeck, E., Bacheler, L., 2008. Determination of clinically relevant cutoffs for HIV-1 phenotypic resistance estimates through a combined analysis of clinical trial and cohort data. J. Acquir. Immune Defic. Syndr. 48, 26–34.
Winters, M.A., Schapiro, J.M., Lawrence, J., Merigan, T.C., 1998. Human immunodeficiency virus type 1 protease genotypes and in vitro protease inhibitor susceptibilities of isolates from individuals who were switched to other protease inhibitors after long-term saquinavir treatment. J. Virol. 72, 5303–5306.
Wirden, M., Simon, A., Schneider, L., Tubiana, R., Paris, L., Marcelin, A.G., Delaugerre, C., Legrand, M., Herson, S., Peytavin, G., Katlama, C., Calvez, V., 2003. Interruption of nonnucleoside reverse transcriptase inhibitor (NNRTI) therapy for 2 months has no effect on levels of human immunodeficiency virus type 1 in plasma of patients harboring viruses with mutations associated with resistance to NNRTIs. J. Clin. Microbiol. 41, 2713–2715.
Xu, L., Pozniak, A., Wildfire, A., Stanfield-Oakley, S.A., Mosier, S.M., Ratcliffe, D., Workman, J., Joall, A., Myers, R., Smit, E., Cane, P.A., Greenberg, M.L., Pillay, D., 2005. Emergence and evolution of enfuvirtide resistance following long-term therapy involves heptad repeat 2 mutations within gp41. Antimicrob. Agents Chemother. 49, 1113–1119.
Yanchunas Jr., J., Langley, D.R., Tao, L., Rose, R.E., Friborg, J., Colonno, R.J., Doyle, M.L., 2005. Molecular basis for increased susceptibility of isolates with atazanavir resistance-conferring substitution I50L to other protease inhibitors. Antimicrob. Agents Chemother. 49, 3825–3832.
Yap, S.H., Sheen, C.W., Fahey, J., Zanin, M., Tyssen, D., Lima, V.D., Wynhoven, B., Kuiper, M., Sluis-Cremer, N., Harrigan, P.R., Tachedjian, G., 2007. N348I in the connection domain of HIV-1 reverse transcriptase confers zidovudine and nevirapine resistance. PLoS Med 4, e335.
Yerly, S., von Wyl, V., Ledergerber, B., Boni, J., Schupbach, J., Burgisser, P., Klimkait, T., Rickenbach, M., Kaiser, L., Gunthard, H.F., Perrin, L., 2007. Transmission of HIV-1 drug resistance in Switzerland: a 10-year molecular epidemiology survey. Aids 21, 2223–2229.
Zachary, K.C., Hanna, G.J., D'Aquila, R.T., 2001. Human immunodeficiency virus type 1 hypersusceptibility to amprenavir in vitro can be associated with virus load response to treatment in vivo. Clin. Infect. Dis. 33, 2075–2077.
Zhang, Y.M., Imamichi, H., Imamichi, T., Lane, H.C., Falloon, J., Vasudevachari, M.B., Salzman, N.P., 1997. Drug resistance during indinavir therapy is caused by mutations in the protease gene and in its Gag substrate cleavage sites. J. Virol. 71, 6662–6670.