

Adherence-resistance Relationships to Combination HIV Antiretroviral Therapy

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Early views on the relationship between adherence and resistance postulated a bell-shaped relationship that balanced selective drug pressure and improved viral suppression along a continuum of adherence. Although this conceptual relationship remains valid, recent data suggest that each regimen class may have different adherence-resistance relationships. These regimen-specific relationships are a function of the capacities of resistant virus to replicate at different levels of drug exposure, which are largely, but not entirely, determined by the impact of mutations on susceptibility of the virus and the impact of the mutations on the inherent ability of the virus to replicate efficiently. Specific patterns of adherence, such as treatment discontinuations, may influence adherence-resistance relationship to combination regimens comprised of medications with differing half-lives. Host genomics that alters antiretroviral drug distribution and metabolism may also impact adherence-resistance relationships. Optimal antiretroviral regimens should be constructed such that there is little overlap in the window of adherence that selects for antiretroviral drug resistance.

Introduction

The development of effective combination antiretroviral therapy (ART) in 1996 transformed HIV from a terminal illness to a chronically manageable disease. Preventing antiretroviral drug resistance is an important goal to preserve the benefits of ART at both the individual and population level. Populations considered at risk for

incomplete adherence—such as the mentally ill, homeless, or drug users—were often considered poor candidates for therapy because of the assumption that even minor lapses in adherence would lead to drug resistance. Given that drug-resistant variants can be spread to others [1], some argued that there was a public health obligation to withhold therapy from such patients [2–6]. These arguments were later echoed in early discussions of the benefits and costs of scaling up ART to resource-limited settings [7–9].

Early views on the relationship between adherence and resistance postulated a bell-shaped relationship that balanced selective drug pressure and improved viral suppression along a continuum of adherence [4]. At low levels of drug exposure (low adherence), there would be insufficient selective pressure for drug-resistance associated mutations to emerge, whereas at high levels of drug exposure, virus replication and presumably viral evolution would be terminated. Although this conceptual relationship remains valid, recent data suggest that each regimen class may have different adherence-resistance relationships [10–15]. These regimen-specific relationships are a function of the capacities of resistant virus to replicate at different levels of drug exposure, which are largely, but not entirely, determined by the impact of mutations on susceptibility of the virus and the impact of the mutations on the inherent ability of the virus to replicate efficiently (replication capacity) [16].

In this review, we will discuss specific antiretroviral drug adherence-resistance relationships as the combination of three phenomena. The first is regimen potency which determines the minimum level of adherence required to fully suppress viral replication. The second is the relative capacity of a virus containing drug-resistance mutations to replicate under drug pressure as compared to the wild-type variant. The third is host genomics that alters antiretroviral drug distribution and metabolism. Finally, we suggest that optimal antiretroviral regimens should be constructed such that there is little overlap in the window of adherence that selects for antiretroviral drug resistance.

Regimen Potency Determines the Minimum Level of Adherence to Prevent Viral Evolution

Durable suppression of plasma HIV RNA to levels below that which is quantifiable with currently available assays (ie, < 50–75 copies RNA/mL) appears to prevent viral evolution and the development of drug resistance [17]. Several earlier reports using objective measures of adherence suggested that near perfect adherence (> 95%) was required to suppress virus in the majority of individuals receiving combination ART containing unboosted protease inhibitors and two nucleoside analogs [18–20]. This high threshold of adherence and the recognition that most common levels of adherence were 70% [21] reinforced public health debates regarding the risks of treating individuals who could not meet this 95% benchmark. These early studies were primarily performed in patients receiving two nucleoside analogues and unboosted protease inhibitors commonly used at the time.

The development of more potent regimens, however, has rendered early regimens consisting of an unboosted protease inhibitor and two nucleoside analogs largely obsolete. Both ritonavir-boosted protease inhibitor-based regimens [22] and non-nucleoside reverse transcriptase inhibitor (NNRTI)-based regimens [23] lead to durable viral suppression in the majority of treatment-naïve individuals and are now considered to be standard-of-care for individuals who are treatment-naïve. Based on the bell-shaped adherence-resistance model outlined above, improved potency of a regimen might be expected to lead to a lower minimum adherence threshold required for full viral suppression and prevention of resistance. Indeed, as outlined below, several studies now find that “complete” viral suppression (ie, < 50–75 copies RNA/mL) is common among individuals taking 70% of either NNRTI or boosted protease inhibitor regimens [24–27]. Although potency contributes to reliable viral suppression at moderate adherence, fitness barriers to resistance (as seen with lopinavir-ritonavir) as well as pharmacokinetic profiles that provide prolonged coverage above the IC_{90} (as seen with efavirenz) also contribute to improved suppression at moderate adherence levels.

Capacity of Resistant Virus to Replicate Under Drug Pressure Determines Minimum Level of Adherence to Select for Drug-resistance Mutations

The selection of a drug-resistant virus requires that randomly generated variants confer a relative growth advantage in the presence of drug. The mechanism for this growth advantage is complex. For most drugs, the initial mutations likely reduce the ability of the drug to bind to its target. Most of the major mutations associated with resistance to the NNRTIs, protease inhibitors, and fusion inhibitors work via this mechanism. Some of the nucleoside-associated mutations that lead to reduced

nucleoside reverse transcriptase inhibitor susceptibility also work via this mechanism (eg, M184V with lamivudine/emtricitabine and K65R with tenofovir) whereas other mutations act to increase excision of the nucleoside analogue from the viral DNA (eg, most of the thymidine analogue mutations) [28]. Regardless of the precise mechanism, it is clear that strong pressures exist in vivo which lead to reductions in the ability of any given drug to inhibit its target protein.

These same mutations often reduce the efficiency of the target protein to support viral replication, at least as it is defined in the absence of drug-pressure (reduced “replication capacity” or, less precisely, reduced “fitness”) [29]. (Fitness refers to the ability of one species to replicate compared with another species in a defined environment; hence, resistant variants are more fit than wild-type variants in presence of drug even though they have reduced replication capacity.) Most of the major mutations selected for by nucleoside analogues, protease inhibitors, and enfuvirtide occur in the active site and therefore reduce replication capacity. In contrast, mutations selected for by the NNRTIs are far from the active site and, therefore, have limited effects on replication capacity.

The complex association between drug-susceptibility and replication capacity are important determinants of the ability for any given drug to select for resistance-associated mutations. It is important to stress, however, that the relationship between how resistance and replication capacity impact the rates at which mutations emerge in vivo have only been carefully studied for NNRTIs and protease inhibitors.

NNRTIs

The NNRTIs are very potent drugs that select for single-point mutations that confer high-level resistance. Because these mutations are distant from the active site of the reverse transcriptase enzyme [30], they do not appear to significantly impact replicative capacity [31–35]. Using clinically derived specimens and in vitro modification of an established phenotypic susceptibility assay, our group recently measured the ability of an NNRTI-resistant virus to replicate at various drug levels compared to a wild-type reference strain and found that the resistant variant replicates more efficiently over a large range of drug-concentrations. By relating these in vitro estimates to the in vivo condition, we estimated that only 2% adherence to the NNRTIs would be necessary to select for and maintain most of the common mutations [16]. Because viral suppression becomes common at greater than 60% adherence to potent NNRTI regimens, the window of adherence that optimally selects for NNRTI resistance is likely between 2% and 60% adherence.

Nucleoside analogues

Most single nucleoside analogue-associated mutations have only a moderate effect on drug-susceptibility, at least

as defined *in vitro*. For example, K65R, L74V, and each single thymidine analogue mutation have only moderate effects on phenotypic susceptibility [36]. These mutations also often reduce replication capacity [36–39]. Hence, the relative ability of a virus containing a drug-resistance mutation to replicate in the presence of drug may not be significantly greater than the capacity of the wild-type variant to replicate in presence of this same drug [37]. This theoretical argument likely explains why these mutations are often not present during early virologic failure and why they accumulate slowly over time [40]. The fact that these mutations do not invariably cause high level phenotypic resistance also likely explains why most nucleoside analogues continue to exert antiviral activity in presence of resistance-associated mutations [41].

The level of adherence required to select for and maintain nucleoside analogue resistance is not known. As these drugs are less potent than other drug classes and as these drugs select for mutations that reduce replication capacity, the level of adherence necessary to select for and maintain a drug resistance mutation is likely to be higher than that observed with the NNRTIs (ie, > 10%), and may be comparable to that observed with unboosted protease inhibitors.

Lamivudine/emtricitabine

Lamivudine and emtricitabine are highly potent drugs that select for a single-point mutation (M184V) that confers high level phenotypic resistance. Hence, it might be expected that the resistant variant would be more fit in the presence of any level of drug-exposure than the wild-type variant. As has been shown in numerous clinical trials and cohort studies, M184V emerges rapidly in the vast majority of patients experiencing an incomplete virologic response to antiretroviral regimen. A precise estimate of the minimum level of adherence required to select resistance for M184V is not available; however, given that these drugs share some similarities with the NNRTIs (ie, they are potent and select for a single highly resistant mutation), it is reasonable to assume that the lower threshold below which resistance does not occur may be very low (ie, < 10%).

Protease inhibitors

The impact of protease inhibitor mutations on drug susceptibility and replicative capacity has been extensively studied. Several consistent observations have emerged. First, although a few inhibitors (eg, nelfinavir and atazanavir) select for common single-point mutations that measurably increase resistance, high level resistance to these and other protease inhibitors requires several mutations [42]. Second, all major protease inhibitor mutations reduce replicative capacity. Third, unboosted protease inhibitor-based regimens are less potent and less effective than boosted protease inhibitor-based regimens at suppressing viral replication [22]. The adherence-resistance profiles for these drugs are generally defined by these properties.

Nelfinavir remains a commonly used protease inhibitor that can not be boosted and is less effective than newer protease inhibitors that can be boosted. The mutation commonly associated with resistance, D30N, does confer only moderate levels of phenotypic resistance and reduces replicative capacity [43,44]. These properties suggest that resistance occurs at moderate to high levels of drug exposure. Using clinically derived samples, we measured the capacity of resistant virus to replicate *in vitro* over a wide range of adherence-derived nelfinavir concentrations and compared with the capacity of a wild-type reference virus and resistant virus isolates to replicate [45••]. In contrast to our experience with NNRTIs, relatively high levels of drug-exposure were needed in order to discern a benefit of the resistance mutations (> 85%). These antiviral characteristics of nelfinavir (ie, lower potency, low effect of single mutations on susceptibility, large effect of these same mutations on replication capacity) all combine to define a window of 85% to 95% adherence as the optimal adherence range for the selection of nelfinavir resistance. The fact that protease inhibitor mutations are uncommon in many patients failing these drugs is consistent with these data [46,47].

Drug resistance to ritonavir-boosted lopinavir is extremely uncommon when these drugs are used in patients who are treatment-naïve [47]. As initially proposed by Kempf et al. [47], this likely reflects the fact that 1) multiple mutations are often required to generate meaningful resistance to lopinavir, 2) these mutations reduce replicative capacity, and 3) the co-administration of ritonavir results in consistently high-levels of lopinavir, making it difficult for the virus to replicate even if some doses are missed [25]. Similar arguments likely apply to other boosted protease inhibitor combinations. Because resistance to boosted protease inhibitors is rare when used as initial therapy [47,48], the window of adherence that selects for resistance is unknown but likely requires erratic, low-level adherence with interruptions of nucleoside analogs over a prolonged periods [49].

Enfuvirtide

Enfuvirtide is similar to lamivudine in several important characteristics: each drug is potent, each drug selects for single-point mutations that result in high-level phenotypic resistance, and each drug selects for mutations that reduce replication capacity. We and others have recently shown that, like lamivudine (and the NNRTIs), resistance to enfuvirtide occurs in nearly all patients failing these drugs. Moreover, resistance to these drugs occurs rapidly and is often present by week 2 and invariably present by week 4 [50]. This rapid emergence occurs even when these changes dramatically reduced fitness (as measured *in vivo* in absence of drug) [51]. Although the precise adherence-resistance profile *in vivo* has not been defined using objective measures of adherence, the virus characteristics outlined above and the consistent observation

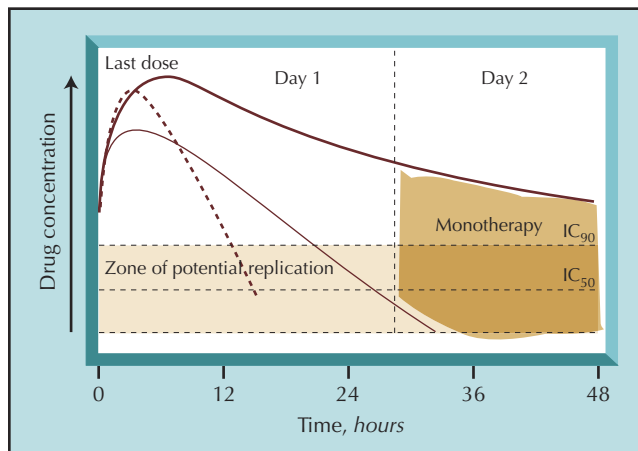


Figure 1. Stopping drugs with different half-lives can lead to periods of monotherapy. Discontinuation of a three-drug antiretroviral regimen leads to declining drug levels according to the half-life of each medication. Longer half-life medications persist after shorter half-life medications are eliminated and lead to periods of monotherapy which can select for drug resistance. (Adapted from Taylor et al. [87].)

that nearly all patients failing enfuvirtide harbor resistant virus strongly argues that the low levels of adherence (ie, < 10%) are capable of selecting for resistant virus.

Patterns of Adherence

Most of our understanding of adherence to HIV ART comes from studies of average percent adherence over a defined period of time. Actual adherence is more complicated as patients may often have periods of high-level adherence followed by periods of low-level adherence. Perhaps the most common form of nonadherence is when all or part of a regimen is completely discontinued. Patients often report selective nonadherence to one or more medications to avoid side effects of particular medications [52]. In resource-constrained regions, intermittent access to antiretrovirals due to logistical or economic barriers may be the most common reasons patients interrupt therapy [53].

Patterns of nonadherence have a large impact on the adherence-resistance relationships in vivo. For example, in the most extreme cases in which therapy is completely interrupted, the pharmacokinetic properties of the interrupted drug become dominant determinants of the rates at which resistance emerges. Discontinuation of antiretroviral regimens with differing half-lives can lead to periods of monotherapy as the levels of shorter half-life medications drop below the therapeutic window while the levels of longer half-life medications remain adequate. This can occur when patients discontinue regimens containing a long half-life NNRTI and two shorter half-life nucleoside analog antiretroviral drugs (Fig. 1). In a study of treatment discontinuations and adherence to NNRTI therapy, Parienti et al. [54•] found that two or more treatment interruptions of at least 48 hours were independently associated with time to the emergence of drug-resistance mutations even after controlling for average adherence.

Spacek et al. [55] found that patient-reported treatment interruptions to NNRTI fixed-dose combination therapy were associated with virologic failure (and presumably drug-resistance) in Uganda. Similarly, Oyugi et al. [56] found that electronically monitored treatment interruptions lasting on average 11.5 days were associated with drug resistance to fixed-dose combination therapy. Based on these findings, some have suggested that individuals discontinuing NNRTI combination therapy should continue the NNRTI component 5 to 7 days before discontinuing the NRTIs. Although this is sound advice in the absence of clinical trial data, it is often difficult to implement this approach in practice given that most treatment interruptions are unplanned and occur in the absence of provider input.

Declining adherence over prolonged periods of time is another common adherence pattern [57,58]. The impact of declining adherence on viral suppression may be related to the duration of viral suppression. Theoretically, initial treatment during high viral burden likely requires higher adherence levels for full viral suppression than later in chronic treatment when viral burden is less. Support for this concept can be found in the recent induction/maintenance clinical trials. For example, monotherapy with lopinavir-ritonavir fails to reliably suppress virus during treatment induction [59] but not when patients switch from a standard regimen to lopinavir-ritonavir after prolonged periods of effective viral suppression [60–62]. Similarly, triple nucleoside regimens may be better able to sustain viral suppression during maintenance than during induction therapy [63,64]. Although there are no direct adherence data to define how adherence thresholds change as a function of changing viral burden on therapy, these induction/maintenance studies suggest that viral suppression may be possible at lower levels of adherence in the setting of reduced viral burden during chronic suppression.

Genomic Determinants of Adherence-resistance Relationships

There is substantial variability in antiretroviral drug levels even among highly adherent patients. Some of this variation may be related to germline polymorphisms in drug metabolizing enzymes or transporters. For example, the NNRTIs are largely metabolized by the cytochrome P450 enzyme CYP2B6 [65,66]. A number of studies have now confirmed an association between CYP2B6 polymorphisms and plasma concentrations of efavirenz and nevirapine [67,68•,69–72]. Homozygosity for the CYP2B6 516T allele is associated with increased NNRTI plasma levels of both efavirenz and nevirapine and a higher incidence of efavirenz-related neurologic side effects. Notably, there are ethnic differences in the prevalence of the CYP2B6 516T allele, with minor allele frequencies reported from the HapMap project of 0.46 for Nigerians, 0.17 for

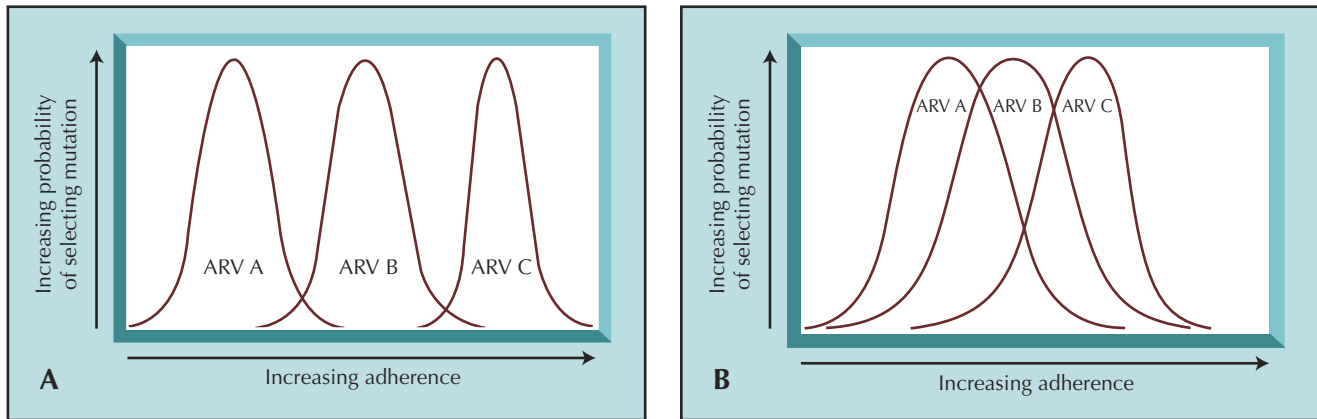


Figure 2. **A**, Nonoverlapping adherence-resistance windows minimize risk of resistance to each antiretroviral (ARV) medication. **B**, Overlapping adherence-resistance windows create range of adherence that select for resistance to multiple medications.

Asians, and 0.21 for Caucasians (www.hapmap.org). Population pharmacokinetic estimates of efavirenz half-lives of 23, 27, and 48 hours for individuals with the *CYP2B6* 516GG, GT, and TT genotypes, respectively, suggests that those with the TT genotype may have prolonged monotherapy after a discontinuation and may, therefore, be at increased risk for development of drug resistance subsequent to treatment discontinuation [73]. Membrane transporters also play an important role in the bioavailability and elimination, as well as the intracellular distribution, of many of the antiretroviral agents. *ABCB1* encodes P-glycoprotein and a synonymous polymorphism in this gene has been associated with a decreased likelihood of virologic failure and decreased emergence of resistant virus with efavirenz, despite no effect on systemic exposure to this NNRTI [68•].

Pharmacogenomic variations in drug distribution may also impact protease inhibitor adherence resistance relationships. The protease inhibitors are substrates for *CYP3A4/CYP3A5*, and there is limited evidence that the *CYP3A5**3 allele influences exposure to saquinavir [74–76]. The formation of the nelfinavir active M8 metabolite is catalyzed by *CYP2C19* and a single nucleotide polymorphism responsible for a splicing defect in *CYP2C19* (681G>A) leads to complete loss of metabolic function and is associated with higher nelfinavir and lower M8 plasma levels [68•,77–79]. P-glycoprotein transport protease inhibitors and polymorphisms in *ABCB1* are associated with increased systemic and intracellular exposure to nelfinavir [80–82]. The association of increased nelfinavir exposure with both the *CYP2C19* and *ABCB1* polymorphisms suggests that patients harboring these variants might lead to different adherence-resistance relationships, such that treatment interruptions could create periods of nelfinavir monotherapy and drug resistance [83].

The intracellular triphosphate levels of NRTIs are determined by the influx of parent drug into the target cell, cellular kinase activity, and efflux of phosphorylated metabolites. The active efflux of NRTI triphosphates is

mediated by the multidrug resistance associated proteins MRP4 and MRP5 [84,85]. In a recent preliminary study, the 4131T>G polymorphism in *ABCC4*, which encodes MRP4, was tentatively associated with lamivudine and possibly zidovudine cellular triphosphate concentrations [86]. Further studies in larger populations will be required to establish the significance of *ABCC4* polymorphisms in determining intracellular levels of the active NRTI metabolites and to examine the importance of these polymorphisms in drug resistance.

Using Adherence-resistance Relationships to Design Regimens to Prevent the Emergence of Resistance

Because each antiretroviral medication has a different adherence range that allows for viral replication and provides sufficient drug pressure to select for drug resistance, it may be possible to combine antiretroviral medications that collectively create a narrow window of adherence capable of selecting resistance. For example, replacing lopinavir-ritonavir with nelfinavir lowers the minimum level of adherence required for full viral suppression. This added viral suppression and moderate range of adherence overlaps with the adherence-resistance window for lamivudine and reduces the rate of lamivudine-resistant failures on lopinavir-ritonavir compared with nelfinavir [22]. If the K65R mutation functions similarly to protease mutations (ie, high levels of adherence are required to select for less fit virus), full viral suppression over a wide range of efavirenz adherence may cover the adherence window required for tenofovir resistance. Furthermore fixed-dose formulation antiretroviral medications may avoid the problem of selective adherence to individual medications and ensures any benefit of adherence-resistance windows. From the perspective of adherence and resistance, the ideal regimen would be a fixed-dose combination regimen where there is no overlap of adherence windows capable of selecting for drug resistance for each medication (Figs. 2A–B).

Conclusions

Anticipated patterns of adherence and resistance have shaped public policy and individual practice in deciding good and poor candidates for therapy. Only recently, however, have sufficient data emerged to more closely define the relationship between adherence and resistance to individual antiretroviral medications. These relationships are best defined for the protease inhibitors and NNRTIs. They are not well defined for nucleoside transcriptase inhibitors, fusion inhibitors, CCR5 inhibitors and integrase inhibitors. Further characterization of levels and patterns of adherence that optimally select for resistance will eventually allow for the design of resilient antiretroviral regimens that remain durably effective across a wide range of adherence behavior seen in diverse populations.

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