Review article

Genetic Barriers to Resistance and Impact on Clinical Response
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Abstract
The development of drug resistance and cross-resistance continues to pose a challenge to successful long-term antiretroviral therapy despite the availability of new antiretroviral agents. The genetic barrier to resistance of a regimen does not directly correlate with its effectiveness. For some regimens with a low genetic barrier to resistance, however, the emergence of only 1 or 2 key resistance mutations may confer drug resistance not only to that regimen but also to other agents, thereby limiting subsequent treatment options. In addition to the genetic barrier to resistance, factors such as efficacy, safety, tolerability, convenience, and adherence must be considered when choosing a regimen.

Introduction
The effectiveness of combination antiretroviral therapy (ART) continues to improve as treatment choices expand with the development of new antiretroviral agents and regimens. However, the emergence of drug-resistant strains of human immunodeficiency virus (HIV) and the resistance of some of these viral mutations to multiple agents or even to entire classes of agents pose some of the greatest challenges to the successful long-term treatment of HIV infection. Accordingly, when selecting antiretroviral regimens, clinicians not only must consider factors such as potency, durability, and the probability of adherence, but also should be aware of resistance patterns likely to be present at the time of virologic failure and the potential impact of these resistance mutations on subsequent treatment options.

There are many potential reasons for virologic failure of an antiretroviral regimen, including suboptimal potency, insufficient adherence to medications (due to any number of factors), negative drug-drug interactions, preexisting drug resistance, or acquired drug resistance. If viral repli-

cation should occur while a patient is taking ART, the evolution of the viral population to acquire a sufficient number of critical drug-resistance mutations to overcome the anti-HIV activity of the drug regimen as a whole is often referred to as the "genetic barrier" to resistance. Stated differently, the genetic barrier refers to the threshold above which clinically meaningful resistance develops, or the ease to which resistance develops, to a drug or a drug class. This threshold is determined by a number of factors, including the number of critical mutations required for loss of activity, the level of preexisting resistance, and the rate of replication of these preexisting resistant strains. It should also be recognized that some mutations, or combination of mutations, might have a greater effect than others. Thus, defining the genetic barrier entails more than simply counting mutations; it also involves determining the effect of single mutations or combinations of mutations on the susceptibility of HIV to the drugs in the regimen. Regimens with a high genetic barrier to resistance, that is, those that require a greater number of critical mutations to render treatment ineffective, include boosted protease inhibitor (PI)- and thymi-

dine analogue-containing regimens.[1-4] Regimens with a low genetic barrier to resistance, those that require fewer critical mutations to render treatment ineffective, may be associated with rapid virologic failure and development of resistance; one recent example of low genetic barrier regimens with high virologic failure rates have been non-thymidine-containing triple nucleoside/nucleotide reverse transcriptase inhibitor (NRTI/NtRTI) combination regimens (eg, abacavir [ABC], lamivudine [3TC], tenofovir [TDF], and didanosine [ddI], 3TC, TDF).[5-8] It should be appreciated, however, that not all regimens with a low genetic barrier have a high rate of early virologic failure; in fact, a number of the most effective antiretroviral combinations contain agents that require only 12 key mutations to confer resistance (eg, NRTI/3TC/efavirenz [EFV]).

Unfortunately, many low genetic barrier agents select for mutations that confer broad class resistance.[9,10] Among the nonnucleoside reverse transcriptase inhibitors (NNRTIs), the selection of the K103N and Y181C mutations cause loss of activity to all currently available NNRTIs. Among the nucleoside analogues, the selection of the K65R mutation causes measurable phenotypic loss of activity to all the NRTIs except zidovudine, and perhaps stavudine (d4T)[11]; the M184V mutation causes loss of activity to both 3TC and emtricitabine (FTC), but has been shown to resensitize the virus to zidovudine (ZDV), d4T, and TDF,[11] as well as to delay the emergence of thymidine analogue mutations (TAMs).[4] Selection of the L74V mutation causes decreased antiviral activity of ABC, ddI, and zalcitabine (ddC), and when the mutated virus is selecting for both L74V and M184V, only the thymidine analogues (ZDV and d4T) and TDF retain susceptibility among drugs in the class.[11] PI-containing regimens are generally believed to have higher genetic barriers to resistance than are NRTIs or NNRTIs, especially when low-dose ritonavir (RTV) is used to boost levels of another PI.[2]

This article examines the factors that may account for the differences in success rates among regimens with low and high genetic barriers to resistance and considers the impact of failure of these regimens on future treatment options. Given the complexity of clinical decision-making among patients with prior treatment and resistance histories, most of the discussion will consider treatment implications of genetic barriers to resistance for initial selection of highly active antiretroviral therapy (HAART).

Low Genetic Barrier to Resistance and High Rate of Virologic Failure
Triple-NRTI/NtRTI Regimens
The quest for simplified regimens with low pill burdens has sparked interest in triple-NRTI combinations that

include the once-daily NtRTI TDF. Novel thymidine analogue-sparing triple-NRTI combinations that include TDF have recently yielded a high rate of early virologic failure in ART-naive patients.

TDF + 3TC + ABC
The triple-combination regimen most commonly associated with early virologic failure has been TDF + 3TC + ABC; the clinical data documenting the failures have been extensively described elsewhere.[12] In general, treatment failures with this combination occur rapidly (usually within the first 3 months of therapy), with the majority of viral isolates having the M184V/I mutation and roughly 50% also containing the nucleoside cross-resistant K65R viral isolate.

Potential reasons for virologic breakthrough with ABC, 3TC, and TDF have centered on negative drug-drug interactions between ABC and TDF, potential pharmacokinetic limitations of once-daily dosing with 3TC and/or ABC, and a low genetic barrier to resistance for the regimen as a whole.[12] Pharmacokinetic data evaluating both the serum and intracellular concentrations of both TDF and ABC (and ABC's intracellular active moiety carbovir-triphosphate) have shown no negative drug-drug interactions.[13,14] Pharmacokinetic data evaluating carbovir-triphosphate and 3TC-triphosphate have shown sufficient drug exposures for once-daily administration[15,16] and clinical data evaluating once-daily ABC and 3TC in combination with EFV have shown good virologic control.[17,18] For example, ESS30009 was a randomized trial of once-daily TDF + 3TC + ABC vs once-daily ABC + 3TC + EFV in 345 ART-naive patients.[17] The mean baseline viral load and CD4+ cell count was 4.63 $\log_{10}$ copies/mL, and 290 cells/microliters (mcL), respectively. In an unplanned interim analysis performed on data from 194 patients, virologic nonresponse (defined as a < 2-log reduction in viral load by week 8 of the study or a 1-log rebound from nadir viral load) occurred in 50 of 102 patients (49%) in the TDF + 3TC + ABC arm compared with only 5 of 92 patients (5%) in the once-daily ABC + 3TC + EFV arm. In addition, viral load < 400 copies/mL was achieved by only 49% of the TDF-treated patients compared with 90% of the EFV-treated patients after 8 weeks (Figure).[17]

Recent data from the Tonus trial (high rates of treatment failure with TDF + 3TC + ABC) have suggested that resistance occurs in a stepwise fashion with M184V first, followed by rapid selection of M184V + K65R.[19] Using selective real-time polymerase chain reaction (PCR) on samples obtained from baseline and weeks 2, 4, and 12, evolution of M184 and M184V + K65R went from 0% for both at baseline to 48% and 4.8% at week 4 and 29% and

57% at week 12, respectively. Although not fully evaluated, the most likely explanation for virologic failure of a TDF+ 3TC + ABC-containing regimen is a limited genetic barrier of each agent in the regimen to the K65R mutation. All 3 agents have decreased phenotypic activity to K65R[11] and thus may allow for the rapid selection of this mutation upon initiation of therapy. The limiting factor to this hypothesis is the fact that all viral isolates contain M184V but not all isolates contain the K65R mutation. One potential explanation for this finding is that K65R may be present but unable to be detected via current resistance testing. Underwood and colleagues[20] recently presented in vitro data that showed that K65R must be present in at least 80% of the viral population in order to be phenotypically detected. If this is true, it is possible for all patients to fail with M184V and K65R mutations; however, only M184V will be reported, especially when present as mixtures with wild-type virus[21]; clonal analysis of minor populations of viral isolates from these treatment failures is currently under way to determine whether K65R was indeed present but not reported.

Phenotypic data from treatment failures of TDF + 3TC + ABC among patients with M184V/I plus K65R have showed retained TDF antiviral activity despite treatment failure with the nucleoside/nucleotide cross-resistant K65R mutation (which has been shown to confer significant loss of activity to TDF).[11] Consequently many clinicians have speculated that TDF can be used in future treatment regimens when K65R is accompanied by the M184V mutation (which has been shown to resensitize TDF). To date, no data exist on the clinical responses to regimens following virologic failure with viral isolates of M184V/I with K65R. In addition, there has been speculation that the K65R mutation causes significant loss of viral replicative capacity (RC), especially in combination with M184V; data from small series that have evaluated RC have shown conflicting results, with some data showing a significantly compromised virus[22,23] and others showing modest loss of RC when compared with wild-type virus.[8] Given the high rates of virologic failure, the regimen of TDF + 3TC + ABC should be avoided.

Recent clinical and in vitro data have suggested that the use of thymidine analogues prevents the development of K65R because TAMs and K65R appear to be mutually exclusive.[8,11,24,25] The impact of having the nucleoside cross-resistant K65R mutation on future nucleoside treatment options has yet to be determined; therefore, treatment options upon failure should include boosted PI- and/or NNRTI combinations.

TDF + 3TC + ddI
Similar to TDF + 3TC + ABC, the combination of TDF + 3TC + ddI appeared to offer a convenient, highly potent, once-daily regimen that could be administered at the same time. However, similar to TDF + 3TC + ABC, early and high rates of virologic failure were reported. In a small pilot study, 24 treatment-naive patients initiated a once-daily regimen of TDF + 3TC + ddI; median baseline viral load and CD4+ cell counts were 4.91 $\log_{10}$ copies/mL and 133 cells/mcL, respectively.[26] By week 12, the median decline in viral load was only 0.61 $\log_{10}$ copies/mL. Genotypic testing in 20 patients who met the criteria for virologic nonresponse (defined as < 2-log copies/mL decline in HIV RNA by week 12) revealed that all had the M184I/V mutation with 10 also having the K65R mutation. Phenotypic testing in 19 patients demonstrated continued susceptibility to TDF in all; however, 5 of the 10 with the K65R mutation had reduced susceptibility to ddI. The precise reason for retained TDF susceptibility and the clinical responses to subsequent regimens containing TDF has yet to be determined. As discussed above, one potential explanation may be the phenotypic evaluation of mixtures of resistant viral isolates with wild-type virus and therefore may skew the results to appear more sensitive than they actually are.[20,21]

Although not fully evaluated, the most likely explanation for treatment failure of this regimen appears to be a low genetic barrier to resistance, as all 3 agents are known to show decreased activity against K65R. However, similar to TDF + 3TC + ABC, resistance analyses revealed that not all patients experienced virologic failure with K65R, and therefore the precise cause of treatment failure is still unknown.

TDF + ddI + EFV
The combination of TDF + ddI + EFV represents a potent and convenient once-daily combination. Clinical data have shown this regimen to maintain viral suppression upon treatment switches among patients with well-controlled HIV infection.[27] The clinical utility of this regimen as initial therapy was recently evaluated in a 3- vs 4-drug treatment strategy study.[28] Patients were randomized to receive either TDF + ddI + EFV or TDF + ddI + 3TC + LPV/r (lopinavir/ritonavir) as initial therapy; the median baseline viral load was 146,000 copies/mL in the 4-drug arm and 143,000 copies/mL in the 3-drug arm; the median CD4+ cell counts were 162 and 195 cells/mm³ in the quad- and triple-drug arms, respectively. The study had to be halted after 3 months when 43% (6/14) of TDF + ddI + EFV-treated patients experienced virologic failure (defined as < 2-log copies decline in HIV RNA at month 3, or either a rebound of >1 log from nadir at month 6, or detectable RNA at month 6 or after) as compared with no patients receiving TDF + ddI + 3TC + LPV/r (0/12). Patients who experienced virologic breakthrough were more likely to have higher viral load measurements and lower baseline CD4+ cell counts at baseline. Among those experiencing virologic failure, 5/6 had the NNRTI-associated G190S/E alone or with K103N and other mutations.

The NRTI-associated L74V mutation was found in virus from 4/6 patients, 2 of whom also selected for K65R. The L74V, K65R and EFV mutations all appeared early in therapy (within the first 3 months).

The precise reason for the alarming rate of treatment failure with TDF + ddI + EFV is unknown. It is of interest that it appears that this regimen is sufficient to maintain viral suppression once viremia is controlled, however insufficient to fully suppress viral replication when used as initial therapy. On the basis of genetic barrier considerations, this regimen should have provided adequate coverage against most preexisting viral mutants. The selection of the NNRTI mutation G190A in most patients in combination with L74V and few K65R mutants, without selection of K103N, suggests a unique pattern of resistance that is selected for by this regimen. To date, few intracellular pharmacokinetic data exist to evaluate whether there is some unexpected drug-drug interaction occurring between TDF + ddI within cells or on the cell wall that could compromise the activity of this regimen. The clinical utility of TDF + ddI + NNRTIs as initial HAART therapy is unknown and should therefore be avoided.

Low Genetic Barrier to Resistance With High Rate of Virologic Success

Other regimens with a low genetic barrier to resistance often achieve a high rate of virologic suppression. EFV plus 3TC-based regimens have become the cornerstone of therapy for many treatment-naive patients, yet each of these antiretrovirals is associated with a low genetic barrier to resistance. Despite this, a number of key studies using these 2 agents as initial ART, in combination with various third antiretroviral agents with varying genetic barriers (ZDV, d4T, TDF, ABC, ddI), have shown good clinical response rates and durable viral suppression with limited development of drug resistance.

TDF + 3TC + EFV

In a 3-year, randomized, double-blind, active-controlled study of TDF vs d4T in 600 ART-naive patients, TDF + 3TC + EFV and d4T + 3TC + EFV proved to be similarly effective in suppressing viral loads in patients treated for 3 years.[29] The final 144-week analysis revealed that a very limited number of patients 47 (15.7%) TDF- and 49 (16.3%) d4T-treated patients experienced virologic failure. EFV and M184V resistance mutations were most common, occurring in 8.3% of the TDF group and 5.8% of the d4T group overall. Through week 144, the K65R mutation was observed in only 8 patients (2.7%) in the TDF arm and 2 patients (< 1%) in the d4T arm. Among patients experiencing virologic failure in the TDF arm by week 96 (n = 36), 8 (24%) had developed the K65R mutation (7

within the first 48 weeks of treatment); no patient acquired this mutation after week 96.[29]

Treatment failures with 3TC + EFV when used in combination with either TDF or d4T are low provided that patients are adherent to therapy and no baseline viral resistance is present. Should viral replication occur while on therapy, both the M184V and K103N mutations are common, whereas the overall risk of developing treatment failure with K65R is rare and more likely to occur within the first year of therapy. Despite the fact that all 3 agents in the TDF + 3TC + EFV regimen have a low genetic barrier to resistance (TDF K65R, EFV K103N, and 3TC M184V), the activity of the regimen as a whole is sufficient to produce high rates of virologic suppression with durable treatment responses. As a result, TDF + 3TC + EFV is listed as a preferred regimen in the Department of Health and Human Services Consensus Panel Guidelines for initial ART among treatment-naive HIV-infected patients.[30]

ZDV + 3TC + EFV

In contrast to TDF, ZDV has shown a wide genetic barrier to resistance with TAMs developing slowly, even when it was used as monotherapy.[31] Regimens containing a thymidine analogue with 3TC or FTC have shown a high genetic barrier to NRTI-associated resistance and a delayed emergence of TAMs in the presence of the 3TC- and FTC-associated mutation, M184V.[3,4] Multiple TAMs confer NRTI cross-resistance, especially when the selected TAM pathway to resistance includes mutations at codons 41, 210, and 215.[6,9,32] The accumulation of TAMs is slow and stepwise after initial virologic breakthrough and this accumulation generally precludes the presence of the mutations L74V or K65R.[4,32] When ZDV is combined with the low genetic barrier agents, 3TC and EFV, good virologic control has been observed; this regimen is often considered to be the "gold standard" of HAART to which other combination therapies are often compared.

When compared with an unboosted PI regimen of indinavir (IDV) + ZDV + 3TC or EFV + IDV, the combination of EFV + ZDV + 3TC was associated with a significantly greater proportion of patients achieving viral loads < 50 copies/mL at 48 weeks in an intent-to-treat analysis (64% in the EFV + ZDV + 3TC group vs 47% in the EFV + IDV group and 43% in the IDV + ZDV + 3TC group).[33] Even among patients in this study with high baseline viral loads (100,000 copies/mL), the EFV + ZDV + 3TC regimen was significantly more effective than the other combinations. The resistance pattern seen among patients experiencing virologic failure on EFV + 3TC + a thymidine analogue commonly includes the selection of M184V and NNRTI

mutations early in virologic failure, with a significant delay prior to the accumulation of TAMs.[29,34]

ABC + 3TC + EFV

Recent studies have also evaluated the once-daily combination of ABC + 3TC + EFV as initial therapy for treatment-naive HIV-infected patients.

In the ZODIAC study (CNA30021), 770 treatment-naive patients were randomized to once- or twice-daily ABC and also received once-daily 3TC and EFV.[18,35] Overall, 66% and 68% of patients in the once-daily and twice-daily arms, respectively, had achieved a viral load measurement < 50 copies/mL by week 48 (via intent-to-treat). Documented virologic failure was rare and occurred in only 10% of those on once-daily ABC and 8% of those on twice-daily ABC. Genotypes could be quantified for 18 patients on once-daily ABC and 20 on twice-daily ABC. There were no significant differences between the study arms in number of patients with treatment-emergent resistance to any drug; the most common NRTI resistance mutations seen in the once-daily treatment arm were M184V (61%) and L74V (31%). When baseline resistance was accounted for, only 1 patient had a documented L74V mutation (7%). Other mutations were rare: K65R was seen in 1 patient, and Y115F and TAMs were each seen in 1 patient in each study arm. As expected, a high proportion of patients with treatment failures in either the once-daily or twice-daily arms had EFV associated mutations (61% once-daily and 70% twice-daily, respectively).

The L74V mutation is rare, but may become more prevalent with common use of ABC/3TC-containing regimens. In contrast to the broad cross-resistance seen with K65R, the L74V mutation alone confers modest loss of antiviral activity to ABC and ddI, but TDF, ZDV, and d4T all remain phenotypically susceptible.[11] However, when combined with M184V, ABC and ddI activity is significantly compromised, leaving the thymidine analogues and TDF susceptible (with ZDV and TDF being hypersusceptible upon phenotype).[11]

The precise impact of L74V on TDF susceptibility has recently been questioned.[36] Data from Gilead's 902 and 907 studies, which evaluated the impact of preexisting L74V mutation on subsequent TDF responses when used in patients with varying treatment histories and various ART regimens, have suggested that this mutation affects clinical responses more than previously expected. Of the 14 patients who developed the K65R mutation, 4 had L74V at baseline. Single genome sequencing was performed and showed that while K65R was reported as undetectable, it was present at baseline in 2 patients and subsequently evolved upon initiation of TDF therapy. Consequently, while TDF may appear susceptible to L74V on phenotype, subsequent treatment with TDF may allow for the selection of K65R if present in small, subclinical quasi-species that are undetected via phenotype. As previously discussed, in vitro data have recently shown that K65R needs to be present in at least 80% of the viral population in order to be detected via phenotype.[20] What is unknown at this time is whether this pattern of subclinical K65R will be present upon first treatment failure when ABC + 3TC is used as initial therapy. The Gilead 902 and 907 studies evaluated patients with extensive treatment and resistance histories in which TDF was added to therapy. How TDF-containing regimens respond, and whether the K65R mutation develops following initial therapy with ABC + 3TC-containing HAART, has yet to be determined.

Regimens With High Genetic Barriers to Resistance

Ideally, it would be preferable to have a regimen that is highly potent, produces durable treatment responses, is well tolerated, and has a wide genetic barrier to resistance. The use of boosted-PI combination therapies appears to meet many of these criteria and has been shown to produce beneficial clinical responses with limited drug resistance upon virologic breakthrough. In addition, boosted PIs appear to prevent the development of mutations to other agents within the ART regimen.[2,37]

d4T + 3TC + LPV/r

To date, the most clinical experience with boosted PIs has been with the fixed-dose formulation product *Kaletra* (LPV/r). Long-term evaluations of treatment-naive patients who received LPV/r in combination with d4T + 3TC as part of the pivotal M98863 study showed no PI mutations upon treatment failure (0 of 51 patients) from genotypes taken between weeks 24 and 108. In contrast,

Table 1: PI and NRTI Resistance Mutations Observed Between Weeks 24 and 108 in Patients Experiencing Virologic Failure in the M98-863 Study[2]

Regimen	PI resistance	3TC resistance	d4T resistance
LPV/r + d4T + 3TC(N = 326)	0/51 (0%)	19/51 (37%)	0/50 (0%)*
NFV + d4T + 3TC(N = 327)	43/96 (45%)	79/96 (82%)	9/96 (9%)

* There was evidence of archival resistance at codon 215 in one patient who was disqualified from the analysis.

43 of 96 patients (45%) who received nelfinavir (NFV) in combination with d4T + 3TC experienced primary PI resistance (Table 1).[2] In addition, treatment with LPV/r produced significantly less resistance to 3TC and to d4T than that observed from NFV-treated patients.

The number of LPV/r-associated mutations present in baseline genotypes of heavily treatment-experienced patients was an independent predictor of virologic response among patients subsequently initiating LPV/r-based regimens.[38] Patients who harbored virus with at least 6 LPV/r-associated mutations at baseline were significantly less likely to attain undetectable viral loads compared with those having fewer LPV/r-related mutations. Each additional LPV/r mutation present at baseline was associated with a 14.5% reduction in the probability of virologic success. In another study of patients with advanced treatment histories (multiple PI failures but NNRTI-naive), baseline phenotypic susceptibility and number of genotypic mutations correlated with clinical response to LPV/r plus EFV and NRTIs.[39] It should be recognized that these results were not absolute and that multiple factors contribute to clinical response; among the 8 patients with baseline LPV susceptibility > 40-fold, 4 patients obtained a viral load < 500 copies/mL and were more likely to obtain sufficient LPV drug concentrations to suppress their individual viral isolates.

ABC + 3TC + FPV/r

Similar to LPV/r, boosted fosamprenavir (FPV/r)-containing regimens appear to produce little PI resistance and prevent the emergence of resistance to 3TC. In the SOLO study, in which ART-naive patients were treated with a backbone of 3TC + ABC twice daily plus either FPV/r once daily or NFV twice daily, none of the patients in the FPV/r arm who experienced virologic failure had primary or secondary PI-resistance mutations, compared with half of the patients with virologic failure in the NFV arm (Table 2).[37] In addition, only 13% of the virologic failures in

the FPV/r arm had the M184I/V mutation vs 69% in the NFV arm. In contrast, in the NEAT study, which assessed a backbone of ABC + 3TC twice daily plus either unboosted FPV or NFV twice daily, PI-associated resistance mutations were seen in 29% of FPV-treated patients and 31% of NFV-treated patients who experienced virologic failure.[40] To date, no data exist on the resistance patterns of FPV/r when administered twice daily as initial therapy in patients with no underlying PI resistance.

Atazanavir-containing HAART

Atazanavir (ATV) has a complex resistance profile that is still being elucidated. In 3 clinical trials of a combined 1015 ART-naive patients, the rate of virologic failure with unboosted ATV was 21%, 24%, and 17%.[41] Among patients experiencing virologic failure on an unboosted ATV-containing initial regimen, 5% to 24% had phenotypic and/or genotypic resistance to ATV. ATV/r has not been prospectively studied as part of initial HAART in treatment-naive patients, and therefore no resistance data are available in this setting.

Older, unboosted PIs are associated with a higher incidence of protease and RT mutations compared with boosted-PI-containing regimens. PI-associated resistance mutations upon initial treatment failure with boosted-PI combination regimens have been limited. Although these data have shown no protease resistance upon virologic failure, recent in vitro data evaluating a new PI have shown viruses with no resistance in the viral protease but mutations in the nucleotide positions within the *gag* gene.[42] Whether these mutational changes in the *gag* genome will subsequently be shown to be clinically relevant and limit future PI use has yet to be determined. Limited data exist on treatment responses among patients who fail boosted PIs because they are often treated with NNRTI-based regimens, thereby limiting evaluation of future PI activity. Among patients with prior treatment failures and underlying protease resistance, the response

Table 2: Incidence of the Emergence of Mutations During Therapy at the First Failure Timepoint in the SOLO Study[37]

	FPV/r Once Daily	NFV Twice Daily	P Value
No resistance mutations	84%	31%	< .001
Primary or secondary PI mutations	0%	50%	< .001
M184I/V	13%	69%	< .001
K65R, L74V	0%	6%	.784

Adapted with permission from MacManus et al. GW433908/ritonavir once daily in antiretroviral therapy-naive HIV-infected patients: absence of protease resistance at 48 weeks. AIDS. 2004;18:651655.

to therapy will depend upon the amount of resistance present, the activity of the other agents in the antiretroviral regimen, and the amount of drug exposure obtained by the individual patient. Therefore, genetic barrier is only one key factor in treatment responses when these regimens are used in clinical practice.

Genetic Barrier: Impact on Clinical Decision-Making

With the exception of non-thymidine-containing triple-NRTI/NtRTI regimens, which should be avoided due to high rates of treatment failure, the decision to use a low- or high-genetic-barrier regimen as initial ART in treatment-naive patients is not definitive and requires careful consideration of a number of key individual patient factors, including treatment history, propensity for being nonadherent, comorbid conditions, and potential for negative drug-drug interactions, among others. If a regimen with a low genetic barrier is initiated and the patient experiences virologic failure, there is a strong potential for development of resistant viral isolates that could limit future treatment options. In contrast, a regimen with a wide genetic barrier (eg, boosted PIs) may provide a potent regimen with good virologic control and limited development of resistance upon virologic failure, but may be compromised by adverse drug events or other treatment-limiting issues (eg, lipid alterations).

Among patients in whom nonadherence may be an issue when initiating ART for the first time, clinicians may choose to initiate once-daily therapy and/or fixed-dose combinations in hopes of minimizing missed doses. If viral breakthrough occurs on an NNRTI-containing regimen with a low genetic barrier, there is a high probability that an NNRTI cross-resistant mutation will occur that will prevent the future use of all currently available NNRTI agents. Should a once-daily regimen be selected, it may be best, if possible, to select a wide genetic barrier regimen that contains a boosted PI because virologic failure will have limited protease and RT resistance. Of the boosted PIs, only FPV/r is FDA-approved for once-daily dosing. Recent data have shown that LPV/r can be administered once daily; however, it has been associated with significantly greater gastrointestinal adverse drug events which could preclude its use.[43] No data exist on the efficacy, safety, or resistance profiles upon treatment failure of ATV/r as initial therapy in treatment-naive patients, although there is no reason to believe that this regimen will not produce limited protease and RT resistance similar to what has been observed with LPV/r and FPV/r.

The selection of the nucleoside backbone for once-daily administration includes ddI + 3TC, TDF + ddI, TDF + FTC

(or 3TC), and ABC + 3TC. Although both TDF + ddI and ddI + 3TC are viable options, it is highly likely that TDF + FTC and ABC + 3TC will be used in a large proportion of patients given the recent FDA approval of these agents in fixed-dose formulations. When either of these combinations has been administered with EFV or boosted PIs, little virologic failure occurs provided that the patient is adherent to therapy and no underlying resistance is present. Consequently, the selection of one of these NRTI backbones for a given patient should be based on which is more likely to be tolerated and adhered to; if there is no obvious preference between the 2 based on these factors, then careful assessment and consideration should be given to which combination will allow for the preservation of better treatment options upon virologic failure. Among the small number of patients who have experienced virologic failure when receiving one of the above treatment options, it appears that ABC + 3TC-containing regimens have a propensity to fail with M184V (and L74V if prior resistance is present at baseline), whereas TDF + 3TC regimens fail with a greater likelihood of having M184V (plus K65R in roughly a quarter of all cases). In vitro genotypic and phenotypic data have shown K65R to be a nucleoside cross-resistant viral isolate that decreases the antiviral activity of all NRTIs except ZDV, whereas L74V in combination with M184V limits the activity of ABC, 3TC, and ddI (also ddC) and thereby preserves TDF and the thymidine analogues. The questions of whether K65R plus M184V causes TDF to retain susceptibility when TDF regimens fail and L74V "masks" underlying K65R mutations that are present in subclinical concentrations, and subsequently cause treatment failure when TDF is initiated, have yet to be fully addressed. In addition, the overall incidence of L74V and K65R in the past has been very limited, and therefore the clinical responses to therapy following the development of these mutations are not well characterized. Consequently, in theory a regimen of ABC + 3TC + a boosted PI in treatment-naive patients appears to provide the most treatment options should viral breakthrough occur; however, only widespread clinical experience and continued clinical research will definitively answer this question.

Should treatment options limit the use of NNRTIs and/or boosted PIs, the use of triple-NRTI/NtRTI-based regimens should include a thymidine analogue in order to prevent the development of the K65R mutation. Although not as potent as NNRTI-based HAART, the fixed-dose formulation product *Trizivir* (ZDV + 3TC + ABC) does provide good antiviral activity with simplified twice-daily dosing, especially among patients with low baseline viral load measurements. In addition, *Trizivir* treatment failures have been shown to produce either wild-type virus or
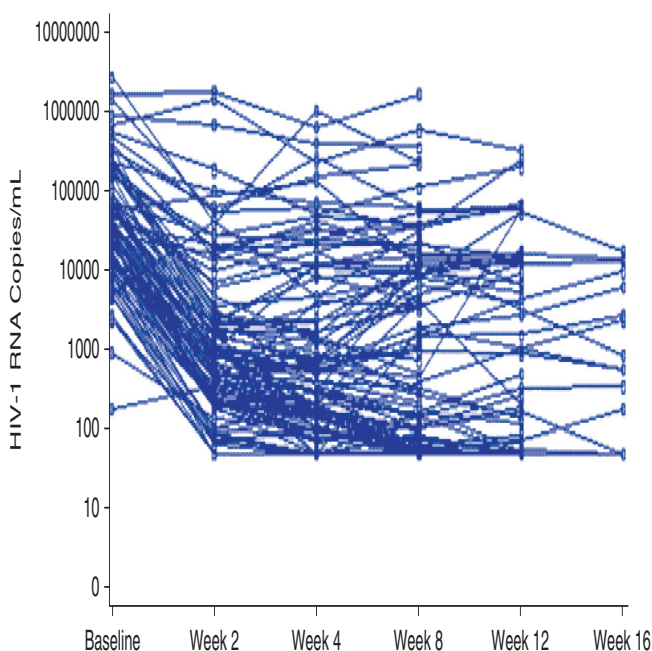
Figure 1
Patients on the TDF + 3TC + ABC regimen in the ESS30009 study demonstrated a high rate of early virologic nonresponse compared with those treated with EFV + 3TC + ABC. [17] Reproduced with permission from Joel E. Gallant, MD.

M184V alone, and the development of TAMs is substantially delayed in this setting, thereby preserving future treatment options.

Among patients with prior treatment experience, it is important to know how much underlying resistance may be present (through detailed treatment histories that include documented resistance testing, if available). The greater the underlying resistance, the greater the chances for lower antiviral responses from the regimen as a whole.

Conclusion

The management of HIV is complicated by a number of critical factors, including drug resistance. Determining whether drug resistance develops upon viral breakthrough will depend upon the level of preexisting resistance, the amount of viral replication, and the genetic barrier of the regimen to resistance. A number of new regimens have been employed for treatment of HIV and have been shown to be highly potent with durable treatment responses despite having low genetic barriers to resistance. However, a number of novel, thymidine-sparing triple-nucleoside-based regimens have experienced high rates of virologic failure with development of cross-resistant viral isolates. Although not fully investigated, it

appears that a low genetic barrier to resistance was the cause of these failures.

The decision to use a specific regimen as initial therapy for HIV must be individually tailored to the patient's lifestyle. Factors such as potential for adherence, low rates of adverse drug events, minimal negative drug-drug interactions, and regimen potency must be taken into consideration. The antiviral activity of many of these newer highly potent regimens is very good, and most patients will experience a beneficial virologic response if they are adherent to therapy and not infected with a resistant viral isolate. Although virologic failure rates are generally low with currently recommended initial regimens, clinicians should carefully consider the genetic barriers to resistance and mutational profiles likely to occur upon virologic failure with each of these regimens when selecting an initial therapy.

Authors and Disclosures

Andrew D. Luber, PharmD, has disclosed that he is a consultant, lecturer, and advisor for GlaxoSmithKline, Vertex, Abbott, and Roche. He has received research grants from GlaxoSmithKline, Vertex, Abbott, and Roche.

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