Assessing Resistance Costs of Antiretroviral Therapies via Measures of Future Drug Options

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The emergence of drug-resistant human immunodeficiency virus (HIV) type 1 in the setting of antiretroviral therapy failure limits the number of drugs available for use in subsequent therapy regimens. To quantify the relative HIV-1–resistance costs associated with various antiretroviral therapy strategies, we developed 2 related measures of future drug options (FDOs) by use of rule-based genotype-interpretation systems. The FDO1 metric assesses the number of drug classes that remain useful; the FDO2 metric assesses the number of drug classes that remain useful and the number of active drugs within each class. Application of these methods is illustrated with data from a randomized study of 3 therapy regimens in nucleoside analog–experienced patients. Each therapy regimen resulted in a unique pattern of drug-resistance (and cross-resistance) mutations. The regimen with the highest virologic failure rate preserved greater future drug options. Quantification of future drug options as an outcome of antiretroviral therapy trials may complement traditional clinical, virologic, and immunologic end points, thereby providing novel insights.

Highly active antiretroviral therapy (HAART) fails to result in complete virus suppression for many patients [1, 2]. The reasons for this failure are often multifactorial and include nonadherence, use of suboptimal regimens with inadequate potency, infection with drug-resistant variants, accelerated drug metabolism, and advanced immunodeficiency [3–5]. As a consequence of incomplete virus suppression, viral replication occurs in the presence of a strong selective pressure, and, after a varying period of time, resistance-associated mutations emerge [6]. Because resistance to 1 drug often confers cross-resistance to other drugs within a class [7] and because the virus and host factors that led to the initial failure often persist during the second regimen, many patients for whom an initial regimen fails often are unable to achieve a durable response to a subsequent “salvage” regimen. High virologic failure rates during salvage therapy are seen even when new therapeutic classes are used [8, 9].

Although drug resistance results in loss of virologic activity and increasing levels of viremia, many patients continue to derive immunologic and clinical benefit from therapy, even after highly resistant variants emerge (as long as therapy is maintained) [2, 10, 11]. Again, the reasons for this phenomenon are multifactorial and include continued partial activity of therapy against the highly resistant variant, reduced viral “fitness” of the drug-resistant variant, and, perhaps, enhanced human immunodeficiency virus (HIV)–specific immune responses [12]. Many patients in clinical practice are now continuing to receive stable therapy, despite ongoing viral replication, because of the continued benefit conferred by partially effective therapy regimens and the risk of rapidly rendering multiple drugs within
each drug class useless when aggressive switch strategies are used. This approach is commonly used, despite evidence that further virus evolution is likely to occur with continued therapy [13]. The extent of reduction in chances of achieving virus suppression (resistance cost) of such a strategy in clinical trials or observational studies is difficult to measure because of the lack of a standardized methodology for quantifying the effect of resistance mutations on future therapeutic options.

There are fewer options for management of virologic failure in resource-poor regions. Access to antiretroviral drugs is increasing for 98% of HIV-infected individuals, who live in resource-poor regions [14, 15]. However, in developing countries, drug options, monitoring tools, and sequential therapeutic schemes are limited. Thus, systematic identification and adoption of simple and effective “therapy option-sparing” strategies are vital.

On the basis of these concerns, we have developed a methodology that quantifies the effect of any given therapeutic strategy on drug resistance. Specifically, we sought to define a metric that quantifies the number of antiretroviral drugs that are likely to be effective, on the basis of genotypic and/or phenotypic resistance measurements at specified time points after study entry, in the context of a study of antiretroviral therapy. Currently available measures of resistance, such as the genotype summary score, make use of genotype-interpretation systems to count the number of drugs in a patient’s regimen to which the patient’s virus is expected to be sensitive [16, 17]. Here, we extend this concept to consider sensitivity to all possible future drug regimens and apply our metric to a recently published randomized clinical study [18]. Our end points, denoted “measures of future drug options” (FDOs), summarize the susceptibility of the viruses from each study regimen to all available drugs within the classes being studied. For example, there are currently 7, 3, and 6 FDA-approved drugs in the NRTI, NNRTI, and PI classes, respectively. Considering these 3 classes of drugs, we might choose to be 17, the total number of drugs (16) plus 1. Thus, the ratio of to will always be <1. Note that this system ensures that the different combinations of NC and ND achieve FDO2 values that are strictly ordered and are determined primarily by the number of susceptible drug classes (NC) and secondarily by the total number of susceptible drugs (ND). In all cases, the resulting FDO2 is an ordinal variable taking no more than 34 values between 0 and 4.

FDO measure 2 (FDO2). FDO2 is determined by 2 components: the number of drug classes that include at least 1 drug to which the patient’s virus is susceptible and the total number of effective drugs within each class. In determining the order of FDO2, the first component is deemed to be more important than the second, which can be used to break ties. FDO2 is calculated as follows: the susceptibility of virus to each drug and the number of drug classes containing at least 1 active drug (NC) is determined as for FDO1. FDO2 is then calculated using the following formula: , where ND is the total number of active drugs, and is a constant that guarantees that always a fraction between 0 and 1.

The constant is defined on the basis of the number of drugs within the classes being studied. For example, there are currently 7, 3, and 6 FDA-approved drugs in the NRTI, NNRTI, and PI classes, respectively. Considering these 3 classes of drugs, we might choose to be 17, the total number of drugs (16) plus 1. Thus, the ratio of to will always be <1. Note that this system ensures that the different combinations of NC and ND achieve FDO2 values that are strictly ordered and are determined primarily by the number of susceptible drug classes (NC) and secondarily by the total number of susceptible drugs (ND). In all cases, the resulting FDO2 is an ordinal variable taking no more than 34 values between 0 and 4.

Statistical methods. Three types of scientific questions may be addressed with the FDO metric, each of which requires unique analytical approaches. First, therapy effects on resistance over some fixed period of follow-up (e.g., 48 or 96 weeks from study entry) may be compared by measuring the FDO end points at the start and the end of the period. Resistance cost (C) is defined as the difference in FDO end points at those 2 time points. Rank-based tests can be used to identify the presence of therapy effect. One concern that arises is how to account for patients for whom resistance-testing data are not available (either because the level of viremia is low or undetectable or because a patient prematurely discontinues therapy). For patients with low or undetectable levels of viremia, one can use the last genotype data available and assume that no additional
mutations have arisen since that time—a reasonable assumption given the limited risk of new resistance associated mutations in patients with durable virologic control during HAART [19]. Patients who have prematurely interrupted therapy may no longer have detectable levels of drug resistance at the end of the period of interest (given the observation that such mutations often wane in the absence of drug pressure). The last genotype data available during active therapy could be used for such patients.

Second, we can compare resistance costs of therapies over variable periods of time—for example, time from start of therapy to virologic failure. The resistance costs associated with virologic failure ($C_{VF}$) can be quantified by calculating the changes in FDO measures from start of therapy to virologic failure. Because of the limited follow-up time and drug potency, virologic failures are observed in only a fraction of patients. Resistance cost $C_{VF}$ is measurable only in patients with observed virologic failure. Our analysis estimates an average resistance cost associated with mean time to virologic failure for patients receiving the same therapy. The special estimation procedure is detailed in the Appendix [20–22]. After obtaining the estimates of resistance costs and their variances, for each therapy arm, we may conduct pairwise comparison between therapy arms, in terms of $C_{VF}$ by use of 2-sample $t$ tests.

Third, because therapies may differ in both effective duration and resistance costs at failure, we propose to compare a duration-adjusted resistance cost measure ($dC_{VF}$) among therapy arms, which is the ratio of resistance cost at virologic failure ($C_{VF}$) to the effective duration of therapy ($T$). The duration-adjusted resistance cost measure may be interpreted as the average amount of FDOs expended per unit of time over the effective duration of therapy. The estimation and testing procedures for $dC_{VF}$ are similar to those for $C_{VF}$.

**Application of FDO in clinical trials.** To illustrate our methods, we used data derived from the AIDS Clinical Trials Group (ACTG) 364 study, a randomized, multicenter, 3-arm clinical trial comparing PI nelfinavir and/or the NNRTI efavirenz in combination with 2 nucleoside analogs in HIV-infected patients with prolonged but exclusive NRTI exposure (median, 5.6 years) [18]. All patients were PI and NNRTI naive at entry. In ACTG 364, 195 patients were randomly assigned to 1 of the 3 therapy arms, denoted as nelfinavir, efavirenz, or nelfinavir plus efavirenz, respectively (all patients also received nucleoside analogs). Our analysis focused on 161 patients for whom baseline genotypic data were available. When a patient’s virus load was confirmed to be $>2000$ HIV RNA copies/mL at or after week 16 (used as the definition of virologic failure for our purposes), a genotypic test was conducted. Eighty patients experienced virologic failure and received a postbaseline genotypic test before study closure. The remaining 81 patients did not experience virologic failure at the time of study closure.

Written, informed consent was obtained from all patients or their guardians, and human experimentation guidelines of the US Department of Health and Human Services and the institutional guidelines were followed in the conduct of this research.

Both FDO1 and FDO2 are used to illustrate the comparison of the resistance costs across therapy arms. In the first step of computing these 2 measures, we use the Stanford Inferred Drug Resistance Score Interpretation Rules (Beta Test) to obtain the binary sensitivity scores to each drug (available at: http://hivdb.stanford.edu) [23]. A cutoff point of 15 was chosen, to distinguish between susceptible and resistant, according to the interpreting rules of the Stanford interpretation system. Any other interpreting system could also be used. This flexibility in defining FDO end points ensures that the most clinically meaningful interpreting system can be used and, conversely, that one can test or compare different interpretation systems using the same genotypic data set.

**RESULTS**

**Genotypic resistance and FDOs at study entry (ACTG 364).** Most of the 161 patients had evidence of significant NRTI resistance at baseline. The most prevalent resistance mutations at baseline (with $\geq 10\%$ prevalence) were NRTI mutations: M41I (58.4%), D67N (36.7%), K70R (36.7%), V118I (26.7%), M184V (57.8%), L210W (34.8%), T215Y (48.5%), T215F (15.5%), and K219Q (9.2%). None of the NNRTI mutations was detected, but 2 minor PI mutations (polymorphisms unrelated to PI responses) were found in the baseline genotypes (L63P [58.4%] and V77I [29.2%]). Mutation patterns did not differ significantly between the therapy arms, with respect to polymorphisms.

Table 1 shows the overall baseline resistance in ACTG 364 patients, in terms of measures of future drug options. In deriving FDO2, $T_{ND}$ was set to be a constant 20. The distributions of baseline FDO1 and FDO2 in this study did not differ much, with FDO2 having a slightly larger range. As expected, the Kruskal-Wallis test results showed no difference in the distributions of these baseline measures across the 3 therapy arms.

Figure 1 shows the time to virologic failure for the 3 therapy arms, on the basis of the 161 patients for whom baseline genotypic data were available. When the study ended, 36%, 44%,

<table>
<thead>
<tr>
<th>Resistance measure</th>
<th>Mean (SE)</th>
<th>Median (IQR)</th>
<th>$P^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>FDO1</td>
<td>2.86 (0.55)</td>
<td>3.3 (2.3–3.3)</td>
<td>.23</td>
</tr>
<tr>
<td>FDO2</td>
<td>3.06 (0.60)</td>
<td>3.5 (2.45–3.55)</td>
<td>.36</td>
</tr>
</tbody>
</table>

**NOTE.** FDO, measures of future drug option (see Methods for an explanation of the difference between FDO1 and FDO2); IQR, interquartile range.

$^a$ Calculated by use of the Kruskal-Wallis rank sum test for equal median.

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and 72% patients in the nelfinavir, efavirenz, and nelfinavir plus efavirenz arms, respectively, had not experienced virologic failure (P = .0005, log rank test). The median time to virologic failure in the nelfinavir arm was ~8.0 months, compared with ~26 months in the efavirenz arm and >34 months in the nelfinavir plus efavirenz arm. The pattern seen in figure 1 is consistent with the data analysis results using all 195 patients [18].

Genotypic resistance at first virologic failure (ACTG 364).
Before performing the resistance costs analyses, we examined the viral genotypic profiles at the time of patients’ first virologic failure during the study. Table 2 shows that viruses isolated from 75% of patients in the efavirenz arm and from 57% of patients in the nelfinavir plus efavirenz arm developed an efavirenz-resistance mutation (K103N and G190A/S) at the time of virologic failure. Viruses isolated from 45% of patients for whom the nelfinavir regimen failed developed a major nelfinavir-resistance mutation (D30N); 20%–30% developed a minor nelfinavir-resistance mutation (M36I and N88D/S). In contrast, viruses isolated from only 2 of 14 patients for whom the nelfinavir plus efavirenz regimen failed developed the D30N mutation (table 2).

Among the 80 patients who experienced virologic failure, viruses isolated from 14 developed the M184V mutation, whereas viruses isolated from 26 patients who had the M184V mutation at baseline reverted to wild type at the first virologic failure. Although there is evidence showing that the reversion to wild type in the consensus pol sequence occurs in patients who discontinue lamivudine therapy [24], it is likely that the M184V persists below assay detection and will affect future options. To address this issue, we did 2 sets of analysis by assuming either that reversion represented true susceptibility or that all mutations observed in past and current genotype testing persisted when calculating the current values of FDO end points, noted as “reversion to wild type possible” and “reversion to wild type not possible,” respectively.

Resistance-costs comparison at week 48. We compared the resistance cost across the 3 study arms over the period from study entry to 48 weeks after entry. If a patient’s week-48 genotype data were missing, we use the last available genotype data to compute the FDO. Of the 161 patients, 98 experienced no virologic failure by week 48 and had only baseline genotype data available (27, 31, and 40 patients in the nelfinavir, efavirenz, and nelfinavir plus efavirenz arms, respectively); another 34 patients had only 1 postentry genotype test at or before week 48 (16, 12, and 6 patients in the nelfinavir, efavirenz, and nelfinavir plus efavirenz arms, respectively); the remaining 29 patients had 2 postentry genotype tests by week 48 (16, 9, and 4 patients in the nelfinavir, efavirenz, and nelfinavir plus efavirenz arms, respectively).

Table 3 shows the mean resistance costs by therapy arms and the P values calculated with the Kruskal-Wallis test (a rank sum test for equivalence in the cost distribution), where $C_{W48}$ and

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Study arm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NFV (n = 59)</td>
</tr>
<tr>
<td>Patients experiencing virological failure</td>
<td>38 (64)</td>
</tr>
<tr>
<td>Time to failure, median months</td>
<td>8.2</td>
</tr>
<tr>
<td>New mutation at failure</td>
<td></td>
</tr>
<tr>
<td>D30N</td>
<td>17 (44.7)</td>
</tr>
<tr>
<td>M36I</td>
<td>12 (31.6)</td>
</tr>
<tr>
<td>N88D/S</td>
<td>8 (21.1)</td>
</tr>
<tr>
<td>M46I/L</td>
<td>4 (10.5)</td>
</tr>
<tr>
<td>K103N</td>
<td>0 (0)</td>
</tr>
<tr>
<td>G190A/S</td>
<td>0 (0)</td>
</tr>
<tr>
<td>L90M</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

NOTE. Data are no. (%) of patients, except where noted. EFV, efavirenz triple therapy; NFV, nelfinavir triple therapy; NFV + EFV, quadruple therapy. Percentages were calculated relative to the total no. of patients in each therapy arm. Percentage of patients with a new mutation was calculated relative to the no. of patients in each therapy arm who experienced virological failure, not the total no. of patients in each therapy arm.

Among the 17 patients, 5 had only the D30N mutation, 8 had both the D30N and M36I mutations, 2 had the D30N and N88D mutations, 1 had the D30N and M46I mutations, and 1 had the D30N, M46L, and N88S mutations.

None of the 2 patients had the K103N mutation, but both had the M36I mutation; 1 of them also had the N88D and L90M mutations.

Among the 8 patients who had the K103N mutation, only 1 developed a protease inhibitor mutation (N88S) as well.

Among the 3 patients, 1 had the G190S mutation without the K103N mutation, and 2 had the G190A mutation with the K103N mutation.
Comparison of resistance costs induced by therapies from entry to 48 weeks after study entry.

Table 3. Comparison of resistance costs induced by therapies from entry to 48 weeks after study entry.

<table>
<thead>
<tr>
<th>Possibility of reversion to wild type, resistance cost end point</th>
<th>Mean resistance cost of therapy, by study arm</th>
<th>NFV</th>
<th>EFV</th>
<th>NFV + EFV</th>
<th>P²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Possible</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C₁₁₀₀₂</td>
<td>−0.046</td>
<td>0.23</td>
<td>0.16</td>
<td>.07</td>
<td></td>
</tr>
<tr>
<td>C₁₁₀₀₂</td>
<td>−0.13</td>
<td>0.27</td>
<td>0.17</td>
<td>.002</td>
<td></td>
</tr>
<tr>
<td>Not possible</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C₁₁₀₀₂</td>
<td>0.18</td>
<td>0.37</td>
<td>0.22</td>
<td>.38</td>
<td></td>
</tr>
<tr>
<td>C₁₁₀₀₂</td>
<td>0.10</td>
<td>0.42</td>
<td>0.24</td>
<td>.20</td>
<td></td>
</tr>
</tbody>
</table>

NOTE: C₁₁₀₀₂ and C₁₁₀₀₂, 48-week changes in resistance cost measures future drug option (FDO) 1 and FDO2, respectively; EFV, efavirenz triple therapy; NFV, nelfinavir triple therapy; NFV + EFV, quadruple therapy.

When reversion to wild type is assumed to be possible, the efavirenz and nelfinavir plus efavirenz study arms both have significantly >0 duration-adjusted resistance cost, in terms of both dC₁₁₀₀₂ and dC₁₁₀₀₂ measures. Of note, the efavirenz arm has significantly higher rate of expending FDO over the effective duration than does the nelfinavir arm (P = .006, for dC₁₁₀₀₂ and P < .0001, for dC₁₁₀₀₂ [2-sample t test]). When reversion to wild type is assumed not to be possible, all therapy arms have significantly >0 duration-adjusted resistance cost, in terms of both dC₁₁₀₀₂ and dC₁₁₀₀₂ measures. There is no possibility of reversion to wild type at virologic failure represents true drug susceptibility. If complete reversion to wild type is assumed to be possible, the resistance cost observed at virologic failure to the nelfinavir regimen is not significantly different from 0, whereas the other 2 therapies have nontrivial resistance costs (table 4). Two-sample t tests show that failure of the nelfinavir regimen leads to significantly lower reductions in FDOs, compared with failure of the efavirenz regimen (C₁₁₀₀₂: mean difference, −0.57; P = .001; C₁₁₀₀₂: mean difference, −0.85; P < .0001) and compared with the nelfinavir plus efavirenz regimen (C₁₁₀₀₂: mean difference, −0.34; P = .03; C₁₁₀₀₂: mean difference, −0.49; P = .004).

Comparison of duration-adjusted resistance costs between entry and time of virologic failure. Because the 3 therapy regimens in ACTG 364 have quite different effective duration and resistance costs, it is of interest to consider both aspects and compare the effective duration-adjusted resistance costs from study entry to time of virologic failure. In table 4, dC₁₁₀₀₂ and dC₁₁₀₀₂ represent the average effective duration-adjusted resistance cost in terms of FDO1 and FDO2, respectively.

Comparison of duration-adjusted resistance costs between entry and time of virologic failure. Because the 3 therapy regimens in ACTG 364 have quite different effective duration and resistance costs, it is of interest to consider both aspects and compare the effective duration-adjusted resistance costs from study entry to time of virologic failure. In table 4, dC₁₁₀₀₂ and dC₁₁₀₀₂ represent the average effective duration-adjusted resistance cost in terms of FDO1 and FDO2, respectively.

Table 4. Comparison of resistance costs and duration-adjusted resistance costs from entry to virologic failure (VF) among AIDS Clinical Trials Group 364 study arms.

<table>
<thead>
<tr>
<th>Cost at VF, study arm</th>
<th>Reversion to wild type possible</th>
<th>Reversion to wild type not possible</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resistance cost</td>
<td>Mean (SE)</td>
<td>P²</td>
</tr>
<tr>
<td>C₁₁₀₀₂</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NFV</td>
<td>0.003 (0.116)</td>
<td>.03</td>
</tr>
<tr>
<td>EFV</td>
<td>0.571 (0.127)</td>
<td>.17</td>
</tr>
<tr>
<td>NFV + EFV</td>
<td>0.342 (0.107)</td>
<td>—</td>
</tr>
<tr>
<td>C₁₁₀₀₂</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NFV</td>
<td>−0.164 (0.127)</td>
<td>.004</td>
</tr>
<tr>
<td>EFV</td>
<td>0.685 (0.133)</td>
<td>.04</td>
</tr>
<tr>
<td>NFV + EFV</td>
<td>0.329 (0.117)</td>
<td>.41</td>
</tr>
<tr>
<td>Duration-adjusted cost</td>
<td></td>
<td></td>
</tr>
<tr>
<td>dC₁₁₀₀₂</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NFV</td>
<td>0.010 (0.016)</td>
<td>.07</td>
</tr>
<tr>
<td>EFV</td>
<td>0.072 (0.016)</td>
<td>.36</td>
</tr>
<tr>
<td>NFV + EFV</td>
<td>0.051 (0.016)</td>
<td>.06</td>
</tr>
<tr>
<td>dC₁₁₀₀₂</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NFV</td>
<td>−0.016 (0.018)</td>
<td>.01</td>
</tr>
<tr>
<td>EFV</td>
<td>0.091 (0.017)</td>
<td>.07</td>
</tr>
<tr>
<td>NFV + EFV</td>
<td>0.047 (0.017)</td>
<td>—</td>
</tr>
</tbody>
</table>

NOTE: C₁₁₀₀₂ and C₁₁₀₀₂, resistance cost at VF in terms of measures of future drug option (FDO) 1 and FDO2, respectively; dC₁₁₀₀₂ and dC₁₁₀₀₂, average effective duration-adjusted resistance cost in terms of FDO1 and FDO2, respectively; EFV, efavirenz triple therapy; NFV, nelfinavir triple therapy; NFV + EFV, quadruple therapy.

a Resistance cost between each triple-drug regimen vs. the quadruple-drug regimen (2-sample t test).

b Mean cost is not significantly different from 0 (each); all other mean costs are highly significantly different from 0 (each P < .0001).
significant difference in the effective duration-adjusted resistance cost between the nelfinavir and the nelfinavir plus efavirenz arms, whereas the efavirenz arm has significantly higher duration-adjusted resistance cost than either of the other 2 therapy arms.

**DISCUSSION**

Many factors need to be considered in determining the therapy success of HAART, primarily the ability of a therapy regimen or strategy to prolong life and prevent disease. However, given the clinical effectiveness of HAART [2, 25, 26], the use of clinical end points and/or death as outcome measures is not feasible in many settings. Instead, the use of surrogate markers is necessary to determine optimal therapy strategies. Accepted surrogate markers include plasma HIV RNA levels and CD4+ T cell counts. In the present report, we describe a surrogate marker that captures the preservation of future therapy options, as measured by the absence of drug resistance [27–29]. Our proposed metric (FDOs) allows for easy quantification of the resistance costs associated with any given strategy. In the present study, we applied the FDO approach to a randomized clinical study of 3 therapy regimens: 1 based on a PI, 1 based on an NNRTI, and 1 based on the combination of a PI and an NNRTI. Because each regimen resulted in a unique pattern of drug-resistance (and cross-resistance) mutations, we showed that important new insights could be provided when the FDO was included as a therapy outcome.

The potential utility of the FDO end point is illustrated by considering the therapy outcomes observed in ACTG 364. In this study of heavily NRTI-experienced patients who added 1 or 2 new drug classes when initiating salvage therapy, the proportion of patients who maintained virus load suppression throughout the study was greatest in those receiving nelfinavir plus efavirenz, intermediate in those receiving efavirenz, and lowest in those receiving nelfinavir. However, as shown in the present study, patients randomly assigned to a regimen including efavirenz had significant reductions in their FDO at the time of virologic failure, whereas patients randomly assigned to receive nelfinavir had a significantly lower reduction in their FDO. Thus, although patients were more likely to experience failure with a regimen containing nelfinavir but not efavirenz, they experienced failure with a virus that retained greater susceptibility to other drugs, including PIs other than nelfinavir and each of the drugs within the NNRTI class. This outcome reflected the observation that 45% of the patients for whom therapy with nelfinavir failed had dominant plasma virus containing D30N as the only primary mutation [30]. This mutation is associated with the preservation of susceptibility to all other PIs [31–33].

There are many clinical studies for which the FDO can be used as an outcome measure. For example, an FDO end point may be particularly useful in investigating approaches to treating patients who experience sustained virologic rebound while receiving HAART. Current guidelines recommend switching to a new therapy regimen soon after the detection of virus, in an effort to avoid accumulation of resistance mutations. Nonetheless, patients who continue to receive a stable antiretroviral regimen after the emergence of drug-resistant virus typically maintain some degree of virus suppression, as well as immunologic and clinical benefits [19, 34]. Although patients who continue receiving a partially suppressive regimen may, in fact, accumulate new resistance mutations, it is also likely that many patients who change therapies will experience virologic rebound and develop resistance mutations while receiving their new regimen. Heretofore, there has been no formal investigation of the costs and benefits of delaying changes in therapy, in part because of a lack of appropriate end points. The proposed FDO measures are tools that can quantify the risk of continued drug therapy after initial virologic failure.

FDO end points might also be useful in determining when therapy-naive patients should start receiving an antiretroviral regimen. Early initiation of antiretroviral therapy is likely to delay disease progression in the short term, but some sequential therapy strategies may exhaust therapy options sooner because of the emergence of resistance when therapy fails. Similarly, the FDO end point may be useful in studies evaluating the benefits and risks of sequential therapy interruptions. Interrupting therapy may be associated with increased resistance in some patients and decreased resistance (or prevention of further virus evolution) in others [12, 35]. The robustness of the FDO metric would allow careful assessment of each of these strategies.

The FDO metric may also be particularly useful in anticipating the evolution of drug resistance in resource-poor regions, particularly where virologic monitoring is unlikely because of costs and the need for sophisticated laboratory infrastructure. The current therapy and monitoring standards in resource-rich countries emphasize frequent monitoring for virologic failure and aggressive adjustment of therapy when viremia is detected [3, 4]. This often leads to a rapid sequence of therapeutic switches after virologic failure. A monitoring strategy driven by CD4 cell counts or clinical signs and symptoms that demonstrate the patient’s immune restoration is more easily implemented in resource-poor regions and may be as effective in preventing disease progression [36]. M.L. and Sombat Thanprasertsuk have proposed a clinical study in Thailand to compare monitoring based on virus load with that based on CD4 cell counts (personal communication). Similarly, Rabkin et al. are initiating a study comparing minimal and more-intensive clinical and laboratory monitoring strategies [36]. Assessment of the resistance cost and the FDOs that remain after clinical and immunologic failure of antiretroviral regimens in
resource-limited settings is essential for comparing the long-
term efficacy of different therapeutic and monitoring strategies.

FDO end points may also play a vital role in international
studies of transmission of HIV infection (i.e., those conducted
among serodiscordant sex partners). The HIV Prevention Trials
Network (HPTN) is planning a randomized study to evaluate
whether immediate therapy of HIV-infected patients with CD4
cell counts of 200–350 cells/μL reduces risk of transmission to
initially seronegative sex partners, compared with deferring
therapy until CD4 cell counts decrease to <200 cells/μL. The
study will be conducted in areas of high HIV prevalence
throughout the world. Although immediate therapy may be
expected to reduce risk of transmission early in the study, eval-
uation of the true clinical benefit of each strategy must take
into account both its long-term impact on transmission rates
and the degree to which transmitted viruses are susceptible to
available drugs. Because only a limited number of therapies are
available in the developing world, transmission of resistant virus
is of especially grave concern. As HPTN investigators have
noted, monitoring of the study should be based not only on
rates of transmission of HIV, but also on the genetic charac-
teristics of transmitted viruses.

There are several caveats to the FDO metric that deserve
comment. First, our approach assumes that no mutations ac-
cumulate during periods of time when virus load is below the
limit of assay detection and, therefore, that no additional resis-
tance costs accrue during this period. This assumption may
be warranted on the basis of small studies of virus evolution
in HIV DNA during virologic suppression [37, 38]. Compar-
ison of the FDO between early and late virologic failures can
serve to test the hypothesis that continued replication in the
presence of drug increases the resistance cost. Second, the resis-
tance cost end points we have proposed quantify the future
drug options available to HIV-1-infected patients on the basis
of analysis of genotypic data. However, it would be straight-
forward to incorporate phenotypic data into the FDO metric.
Third, the FDO metric can rapidly accommodate new drugs
as they are developed. For example, for highly drug-experienced
patients, the selection of drugs in a salvage regimen that in-
cluded the newly available fusion inhibitor enfuvirtide could
be optimized by FDO analysis. Finally, the FDO can incorpo-
rate changes in our understanding of pathogenesis, drug resistance,
and susceptibility. Mutational interactions, which may include
increased susceptibility to some drugs on the basis of resistance
to others, may be incorporated into FDO strategies and eval-
uation [39–42]. To compare antiretroviral drug strategies and
regimens, the FDO metric is an analytical tool to define new,
additional outcomes in clinical trials. For the clinician, the FDO
approach may offer a method to synthesize resistance informa-
tion, to optimize sequential drug regimens in the long-term
therapy of HIV infection.

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APPENDIX

Let \( T \) denote the random time from entry to virologic failure
of a patient, and let \( U \) denote the random censoring time,
assumed to be independent of \( T \). Notice that \( T \) is observable
only if \( U > T \). We may denote the random resistance cost ac-
cumulated during period \( T \) as \( C_{\text{res}} \), which is measurable only
if the failure time \( T \) is observed. For each patient, the observed
information is the observed time \( X \), which is the minimum of
\( T \) and \( U \), an indicator \( D = 1 \) if \( X = T \), 0 if otherwise, and
the resistance cost \( C_{\text{res}} \) if \( D = 1 \). We may assume that the cost
accumulation depends on the failure time \( T \) in the same way
for all patients receiving a particular therapy. Averaging over
all possible values of failure time \( T \) leads to a common distri-
bution of \( C \), whose mean \( \mu \) is of interest.

With a sample of size \( n \), \( \mu \) can be estimated as follows [22]:
\[
\hat{\mu} = \sum_{i=1}^{n} D_i C_{\text{res}}(X_i)/\hat{G}(X_i),
\]
where \( \hat{G}(\cdot) \) is the Kaplan-Meier estimator of \( G(\cdot) \) based on
data \((X_i, D_i), i = 1, \ldots, n \). Thus, we estimate the mean
resistance cost by computing a weighted average of the observed
resistance costs, where the weight is the inverse probability of
not being censored at the observed time.

Furthermore, let \( \hat{S}(\cdot) \) denote the survival function of \( T \), and
let \( \hat{S}(\cdot) \) denote the corresponding Kaplan-Meier estimator based
on data \((X_i, D_i), i = 1, \ldots, n \). Define
\[
H(V(X)) = \frac{1}{nS(X)} \sum_{i=1}^{n} D_i I(X_i \geq X) \hat{G}(X_i),
\]
where the variable \( V \) can be \( C_{\text{res}} \) or \( C_{\text{res}}^{-1/2} \). Then the variance of
\( \hat{\mu} \), \( \sigma^2 \) can be estimated by the formula
\[
\hat{\sigma}^2 = \frac{1}{n} \left[ \sum_{i=1}^{n} D_i (C_{\text{res}} - \hat{\mu})^2 \hat{G}(X_i) \right]^{1/2} + \frac{2}{n} \left[ \sum_{i=1}^{n} D_i (C_{\text{res}} - \hat{\mu}) (\hat{G}(X_i) - \hat{H}(C_{\text{res}} X_i)) \right].
\]

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