Residual Activity of Two HIV Antiretroviral Regimens Prescribed without Virological Monitoring

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Virological residual activity (VRA) denotes the degree of HIV RNA suppression achieved by antiretroviral therapy in the presence of resistant virus. This concept is particularly important in resource-limited settings, where rapid switching after detection of virological failure may not be feasible. Using data from the NORA trial, we estimated VRA for two regimens—zidovudine-lamivudine-abacavir (ZDV-3TC-ABC) and zidovudine-lamivudine-nevirapine (ZDV-3TC-NVP)—and related this to the phenotypic drug sensitivity of the component drugs in the two regimens. Plasma samples at weeks 0, 48, and 96 were retrospectively assayed for HIV-1 RNA, and genotypic/phenotypic resistance testing was performed if HIV-1 RNA exceeded 1,000 copies/ml. Virological residual activity (VRA) was defined as the difference between \( \log_{10}(\text{HIV RNA}) \) at week 48 or 96 and week 0 and related to 50% inhibitory concentration (IC\(_{50}\)) relative to wild-type virus for ZDV and ABC (fold change [FC]). Twenty-seven samples in the ZDV-3TC-NVP group and 56 in the ZDV-3TC-ABC group contributed to the analysis. Mean VRA was significantly higher in the ZDV-3TC-ABC group than in the ZDV-3TC-NVP at week 48 (1.62 versus 0.90) and week 96 (1.29 versus 0.78). There was a weak and nonsignificant relationship between VRA and ZDV FC, with VRA decreasing by 0.1 \( \log_{10} \) copies/ml per 2-fold increase in ZDV. The association with ABC FC was much stronger, with a marked reduction in VRA occurring at ABC FC values greater than approximately 2. This information should be considered in future treatment guidelines relevant to resource-poor settings.

Rollout of HIV antiretroviral therapy (ART) in resource-poor settings is characterized by two major differences to that in resource-rich countries: first, few drug combinations are available, and second, there is limited capacity for virological monitoring (HIV RNA viral load, drug resistance testing) of treatment (15). Consequently, patients may experience prolonged periods of undiagnosed viremia on therapy, which can result in immunological compromise via depletion of CD4 count (20) and an increased risk of transmission (28). However, there are few data on the complex and dynamic relationship between evolving drug resistance and the level of viremia (25).

We refer to the degree of HIV RNA suppression achieved by antiretroviral therapy in the presence of virus that is resistant to one or more drugs in the regimen as “virological residual activity” (VRA). At least two factors are involved: a direct antiviral effect of therapy and the maintenance of drug-associated mutations that are detrimental to the fitness of the virus in the absence of drug (10). VRA has been estimated mainly from selective (partial) treatment interruption studies by observing the short-term increase in HIV RNA after discontinuation of one or more antiretroviral drugs while maintaining the other drugs in the current regimen (4, 10, 12, 31). The duration of follow-up in most of these studies has been too short to observe the impact of the reversion of drug-associated mutations on VRA.

Another approach is to compare HIV RNA level in a baseline (pretherapy) sample with that in a later sample in which high-level viral resistance is observed or assumed. Such analyses were performed in early studies of monotherapy and dual therapy (2, 11, 13, 24, 27) but are generally no longer possible because of the current practice of switching therapy in early virological failure (17). An exception is studies in resource-limited settings where access to concurrent HIV RNA testing may not be available but where plasma samples can be stored for retrospective analysis.

One such study is NORA, a randomized trial in Uganda that compared abacavir (ABC) with nevirapine (NVP) in combination with zidovudine-lamivudine (ZDV-3TC). Nevirapine demonstrated short-term virological and immunological superiority over abacavir, although this was not reflected in clinical outcomes (22). Here, we have estimated VRA for the two regimens used in NORA and related it to the phenotypic drug sensitivity of the component drugs.

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MATERIALS AND METHODS

DART was a randomized trial in Uganda and Zimbabwe comparing clinically driven monitoring versus laboratory (CD4/hematology/biochemistry) and clinical monitoring in HIV-infected adults initiating ART with <200 CD4 cells/mm\(^3\) (9). NORA was a randomized double-blind trial conducted in the two Ugandan centers as a substudy within DART (8, 22, 23). Six hundred participants were randomly allocated in a 1:1 ratio to receive zidovudine-lamivudine (ZDV-3TC)
TABLE 1. Selection criteria

<table>
<thead>
<tr>
<th>Parameter</th>
<th>No. of observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZDV-3TC-ABC</td>
<td>ZDV-3TC-NVP</td>
</tr>
<tr>
<td>Wk 48</td>
<td>Wk 96</td>
</tr>
<tr>
<td>No. of patients randomized</td>
<td>300</td>
</tr>
<tr>
<td>Exclusion of data after STI onset</td>
<td>300</td>
</tr>
<tr>
<td>No. of patients on original regimen</td>
<td>245</td>
</tr>
<tr>
<td>HIV RNA result available at baseline and at wk 48/96</td>
<td>240</td>
</tr>
<tr>
<td>HIV RNA of ≥1,000 copies/ml</td>
<td>50</td>
</tr>
<tr>
<td>Phenotypic result available</td>
<td>35</td>
</tr>
<tr>
<td>≥1 major IAS mutation</td>
<td>30</td>
</tr>
<tr>
<td>Exclusion of highly influential observations</td>
<td>29*</td>
</tr>
<tr>
<td>≥1 major NNRTI mutation</td>
<td>NA</td>
</tr>
</tbody>
</table>

* Most changes from the original regimen were due to substitution of zidovudine with lamivudine, abacavir with tenofovir, or nevirapine with tenofovir. Seven patients started second-line therapy, based on lopinavir-ritonavir.

b Excluding 3 samples with undetectable HIV RNA at baseline.

c Mainly accounted for by insufficient sample material.

d Restriction applied to exclude virological failure likely due to nonadherence.

e Outlying observations which strongly influenced model fit (details in Table 2).

f Restriction applied to allow simplification of statistical model.

g, samples included in the final model.

h NA, not applicable.

i STL, structured treatment interruption.

RESULTS

Baseline characteristics in NORA have been described elsewhere (8). In summary, 72% of the participants were female, median age was 37 years, 18% had WHO stage 4 disease, and median CD4 count was 99 cells/mm³, similar to the characteristics of those included in this subanalysis. The rate of virological failure was higher for ZDV-3TC-ABC than for ZDV-3TC-NVP (22). A description of resistance findings and estimates of VRA are presented separately for the two regimens. However, the M184V mutation was ubiquitous in both groups and resulted in high-level phenotypic resistance to lamivudine (median of >58 FC, range of 11 to >147 FC).

ZDV-3TC-NVP. Twenty-seven observations met the inclusion criteria in the zidovudine-lamivudine-nevirapine (ZDV-3TC-NVP) group (Table 1). Fifty-four percent (6/11) of the samples had one or more thymidine analogue mutations (TAMs) at 48 weeks (median of 2), compared with 88% (14/16) of samples at 96 weeks (median of 4). All but one sample had ≥1 major nonnucleoside reverse transcriptase inhibitor (NNRTI) mutation, the most frequent individual mutations being G190AS, K103N, and Y181C/I. Because of the uniformly high level of phenotypic resistance to nevirapine in samples with NNRTI mutations (median of >55 FC, range of >44 to >71 FC), the one sample lacking these mutations was excluded from further analysis, and VRA was related to zidovudine FC only (26 observations, 6 patients with observations at both 48 and 96 weeks). The median (interquartile range [IQR]) FCs...
Mean (standard deviation [SD]) HIV RNA was 5.18 (0.78) \log_{10} \text{ copies/ml} at baseline, 4.45 (0.68) \log_{10} \text{ copies/ml} at week 48, and 4.40 (0.55) \log_{10} \text{ copies/ml} at week 96. The maximum value of HIV RNA at either week 48 or 96 was 5.34 \log_{10} \text{ copies/ml} (219,000 \text{ copies/ml}). Mean VRAs were similar at week 48 (0.90 [SD, 0.78] \log_{10} \text{ copies/ml}) and week 96 (0.78 [SD, 0.80] \log_{10} \text{ copies/ml}). VRA was negative—that is, HIV RNA at week 48/96 exceeded baseline value—for 27% (7/26) of the observations. The association between VRA and zidovudine FC was weak and nonsignificant (Fig. 1a), with VRA decreasing by an estimated 0.09 (95% confidence interval [95% CI], −0.02 to 0.21) \log_{10} \text{ copies/ml} per 2-fold increase in zidovudine (P = 0.12).

ZDV-3TC-ABC. A total of 56 observations on 49 patients (i.e., 7 patients had observations at both 48 and 96 weeks) met the inclusion criteria in the zidovudine-lamivudine-abacavir (ZDV-3TC-ABC) group (Table 1). The only abacavir-associated mutation observed, other than M184V, was K65R in a single patient at week 96. Sixty-six percent (19/29) of the samples had one or more TAMs at 48 weeks (median of 2), which increased to 93% (25/27) of samples at 96 weeks (median of 3). The median (IQR) FCs for abacavir were 0.7 (0.4, 1.0), 1.9 (1.5, 3.0), and 2.4 (1.4, 3.3) at weeks 0, 48, and 96, respectively; the corresponding values for zidovudine FC were 0.9 (0.6, 1.3), 1.5 (1.1, 3.9), and 3.6 (1.6, 5.0).

Mean (SD) HIV RNA was 5.64 (0.58) \log_{10} \text{ copies/ml} at baseline, 4.06 (0.69) \log_{10} \text{ copies/ml} at week 48, and 4.30 (0.75) \log_{10} \text{ copies/ml} at week 96. The maximum value of HIV RNA at either week 48 or week 96 was 5.33 \log_{10} \text{ copies/ml} (211,000 \text{ copies/ml}). Mean VRA was 1.62 (0.72) \log_{10} \text{ copies/ml} at week 48 and 1.29 (0.70) \log_{10} \text{ copies/ml} at week 96. VRA was negative for only 2% (1/56) of the observations.

VRA was inversely related to both abacavir FC and zidovudine FC (Fig. 1b and c). As expected, due to genotypic cross-resistance, abacavir FC and zidovudine FC were strongly correlated (Fig. 2) (Pearson correlation coefficient, 0.55; 95% CI, 0.33 to 0.71). The best-fitting model indicated a linear effect of zidovudine FC on VRA (decrease of 0.11 [95% CI, 0.02 to 0.21] per 2-fold increase, P = 0.01), similar to that observed for the ZDV-3TC-ABC group. However, a cubic transformation of abacavir FC gave a statistically superior fit compared with a linear model (P = 0.02). Graphical representation of this association indicates that the activity of abacavir changes little for FC values less than approximately 2 but falls rapidly thereafter (Fig. 1b). Figure 1c shows the analogous plot for zidovudine FC, at selected values of abacavir FC.

**DISCUSSION**

A striking finding in NORA was the observation of a maximum HIV RNA of 219,000 copies/ml among patients on antiretroviral therapy compared with a pretherapy baseline median HIV RNA of 284,000 copies/ml (23). This suggested a large VRA effect and motivated a detailed analysis of this measure at the individual patient level. VRA was estimated by comparing HIV RNA values after 48 and 96 weeks of therapy with pretherapy values, analogous to the approach used in some studies of monotherapy and dual therapy. Estimates of
noted that the maximum abacavir FC observed in our study incrementally greater the higher the value of abacavir FC. It is ear effect of abacavir FC, with the reduction in VRA being association (14). An important finding is the apparent nonlin-
"true" abacavir FC may be even stronger than the empirical

FIG. 2. Correlation between abacavir fold change (FC) and zidovu-
dine FC. Circles, week 48; squares, week 96.

VRA from these and other studies (6) for the antiretroviral
drugs used in NORA (zidovudine, lamivudine, nevirapine,
abacavir), either as single agents or in combination, are avail-
able in the supplemental material.

Because of uniformly high-level resistance to lamivudine
and nevirapine caused by single point mutations, we were unable to characterize the effects of these drugs. However, the studies with results shown in Table S1 in the supplemental material imply that there was likely to have been no or little VRA from nevirapine whereas lamivudine may have resulted in an approximate 0.5-log10 reduction (~3-fold) in HIV RNA plasma concentration. As VRA reflects the effect of the regimen as a whole, the estimates for zidovudine and abacavir should be interpreted as being superimposed upon this effect. Furthermore, there may be an additional indirect effect, since the continued use of lamivudine maintains the M184V muta-
tion, which partially reverses zidovudine resistance (1).

The relationship between zidovudine FC and VRA was weak in both the ZDV-3TC-ABC and the ZDV-3TC-NVP group, with a doubling of ZDV FC predicting a reduction in VRA of approximately 0.1 log10 copies/ml. This observation was not unexpected, as zidovudine is a relatively nonpotent drug, producing a maximum HIV RNA reduction of approximately 0.5 log10 copies/ml when given as monotherapy (2, 11, 13, 27), and has no discernible antiviral effect with a highly mutated virus. The latter observation is at odds with in vitro data showing that TAMs impair viral fitness (21), although this may be countered partly by the development of compensatory mutations (5).

In contrast with zidovudine, we observed that the level of abacavir phenotypic resistance was an important determinant of VRA, underscoring that VRA is not an absolute phenomenon but depends on the extent of resistance in a particular viral isolate (9). Given the considerable experimental variabili-
ty in the estimation of FC, the association between VRA and "true" abacavir FC may be even stronger than the empirical association (14). An important finding is the apparent nonlin-
ear effect of abacavir FC, with the reduction in VRA being incremen
tally greater the higher the value of abacavir FC. It is noted that the maximum abacavir FC observed in our study (5.2, excluding the sample with the 69 insertion complex) is considerably lower than those described elsewhere for treat-
ment-experienced patients (19).

A key objective in clinical research has been the identifica-
tion of cut-points for phenotypic resistance assays that signify when the effect of a drug ceases to exert meaningful clinical activity (3, 29, 30). However, threshold effects often lack bio-
logical plausibility, and arbitrary statistical rules underlie se-
lection of cut-points. We also note that very large data sets are required to reliably identify the precise form of relationships where both the predictor (FC) and outcome (HIV RNA) vari-
ables are measured imprecisely, particularly when attempting to separate the effects of individual components of a combi-
nation drug regimen. Lanier and colleagues used an add-in design to examine the effect of abacavir on virological response at 4 weeks (19). Using the statistical technique of recursive partitioning, they identified Antivirogram FC cut-points of 3.2 and 7.5, although examination of the empirical data (Fig. 3B in their paper) casts doubt on the reliability of these values; for example, there were only 10 samples with abacavir FC values above 7. Winters and colleagues sought to identify cut-points by fitting linear regression models to a large, combined clinical trial and cohort data set, on the basis of loss of drug activity relative to that for wild-type virus, using HIV RNA response at 8 weeks (29, 30). They estimated 80% loss of abacavir activity at an FC value of 3.5, although this was a predicted, rather than a directly measured, FC value based on a genotypic algorithm. Our analysis suggests an appreciable loss of abacavir antiviral activity at FC values higher than approximately 2.

It should be emphasized that the association between VRA and FC could be estimated only for subjects with HIV RNA greater than 1,000 copies/ml (3.0 log10 copies/ml), the technical limit for reliable resistance phenotyping. Thus, for a subject whose baseline HIV RNA was 4.5 log10 copies/ml, the maxi-

mum observable VRA was 1.5 log10 copies/ml. This phenom-

enon, combined with variability in the measurement of HIV RNA, explains in large part the strong relationship between VRA and baseline HIV RNA. To explore potential bias, we performed a simple simulation study in which we refitted mod-
els after imputing FC values for samples with HIV RNA less than 1,000 copies/ml; these were assumed to harbor no signif-
icant resistance mutations and to have FC distributions that mirrored those of pretherapy samples. This resulted in regres-

sion lines between VRA and observed FC value which were shifted upwards and more steeply negative (not shown). How-

ever, from a clinical standpoint, our empirical analysis may be the most relevant since subjects with low HIV RNA values are not generally considered for treatment switches.

The usefulness of VRA in resource-rich settings is most apparent in helping to guide the selection of salvage regimens in patients with a highly resistant virus. However, its clinical relevance is arguably more pertinent in resource-limited set-
tings, where rapid switching after detection of virological fail-
ure may not be feasible because of the lack of concurrent viral load monitoring and limited access to second-line and subse-
quent regimens (15). The PLATO study of patients with three-
class virological failure found that CD4 count tended to in-
crease if current HIV RNA was less than 4.0 log10 copies/ml or if VRA exceeded 1.5 log10 copies/ml (20). In the ZDV-3TC-
NVP group, average VRA was only 0.8 log10 copies/ml (largely
independent of the level of resistance to zidovudine), and HIV RNA was actually higher at week 48/96 than at baseline for 27% of paired samples. This suggests that there is likely to be no or limited clinical benefit in continuing this regimen following virological failure. In contrast, ZDV-3TC-ABC demonstrated more-potent virological activity in the presence of resistance, with mean VRAs of 1.7 and 1.2 log<sub>10</sub> copies/ml at weeks 48 and 96, respectively.

The key determinant of VRA, from our analysis, appears to be the level of resistance to abacavir. Many viral isolates remained susceptible to this drug, although this proportion is dramatically the proportion of patients remaining virologically suppressed on the population distribution of HIV RNA, particularly the proportion of patients remaining virologically suppressed by time on therapy, is critical to guide programmatic decisions. Such analyses of the regimens used in the DART trial are ongoing.

ACNOWLEDGMENTS


Hertogs, K., et al. 1998. A rapid method for simultaneous detection of phenotypic resistance to inhibitors of protease and reverse transcriptase in recombinant human immunodeficiency virus type 1 isolates from patients


