Short-Term Measures of Relative Efficacy Predict Longer-Term Reductions in Human Immunodeficiency Virus Type 1 RNA Levels following Nelfinavir Monotherapy

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We calculated the relative efficacy of treatment, defined as the rate of decline of virus levels in plasma during treatment relative to the rate of decline during highly potent combination therapy, in human immunodeficiency virus type 1 (HIV-1) patients treated for 56 days with different doses of the protease inhibitor nelfinavir. Relative efficacies based on the rate of decline of HIV-1 RNA levels in plasma over the first 14 to 21 days correlated with drug dose and viral load reduction by day 56. Calculation of relative treatment efficacies over the first 2 to 3 weeks of treatment can allow rapid assessment of new antiretroviral agents and dosing regimens, reducing the need to keep subjects in clinical trials on monotherapy for prolonged periods of time. Relative efficacy may also serve as a measure of treatment efficacy in patients in initiating established therapies.

The development of active and potent inhibitors of human immunodeficiency virus type 1 (HIV-1) reverse transcriptase (RT) and protease (Pr) has drastically changed the natural history of HIV-1 infection. When RT and Pr inhibitors are given in combination, levels of HIV-1 RNA fall dramatically to below the level of detection in many patients (12, 13, 29). Reductions in plasma viremia are generally accompanied by increases in CD4+ T-cell counts (6, 7, 15, 17, 19, 22), improved lymphoproliferative responses (1, 34, 36, 38), and a decrease in the number and severity of opportunistic infections (10, 24, 28). In areas where combination therapies are available, morbidity and mortality due to HIV-1 infection have been significantly reduced (5, 16).

Despite this progress, there are still substantial challenges facing the development of novel antiviral therapies. Adherence to treatment regimens has proven to be difficult given the combination of complicated regimens, frequent dosing intervals, high pill burden, and significant short-term adverse events. In addition, reduced susceptibility to these agents due to the selection of and emergence of viral resistance in vivo has resulted in less-than-optimal therapeutic outcomes in some populations (2, 8, 20, 39). Selection for drug resistance may be related to suboptimal suppression. Recent data suggest that even in highly motivated patients in clinical trials, current regimens do not completely control viral replication (35, 41). Thus, new potent agents need to be developed. Furthermore, to treat patients infected with drug-resistant strains, it will be necessary to develop new compounds that target not only the constitutive enzymes, RT and Pr, but also other components critical to HIV-1's ability to complete its life cycle in vivo, such as HIV-1 integrase (33) and regions of gp120 and gp41 responsible for fusion and entry (18). To be successful clinically, these drugs will need to be easy to take and active against resistant strains of HIV-1. In the era of combination therapy, phase I/II testing becomes challenging.

Prior to the understanding of HIV-1 replication dynamics in vivo and the increased appreciation of the hazards of monotherapy with any agent, the evaluation of the antiviral activity of an agent was usually accomplished by phase I/II studies of prolonged monotherapy. Given that treatment with monotherapy can select for the emergence of drug-resistant viruses and that combination therapy is the treatment standard, new approaches to the assessment of antiviral activity of a particular drug at a particular dose are urgently needed.

Here we propose the use of a mathematically derived factor, “relative efficacy,” which we define as the rate of decline of HIV-1 RNA levels in plasma following treatment with the new antiviral drug divided by the rate of decline following highly potent combination therapies. By retrospectively analyzing results from the phase I/II studies of nelfinavir mesylate, an inhibitor of HIV-1 Pr (37), we have shown that early measures of relative treatment efficacy can predict results of up to 2 months of monotherapy. We believe this represents a straightforward and practical method for assessing a new drug’s antiviral activity at a dose that can avoid prolonged periods of unacceptable exposure to monotherapy.

MATERIALS AND METHODS

Patient characteristics. This study reanalyzes data from Markowitz et al. (21) on thirty Pr inhibitor-naive chronically HIV-1-infected subjects who were assigned to one of six nelfinavir-dosing regimens: 500 mg twice a day (BID) (1,000 mg/day), 600 mg BID (1,200 mg/day), 750 mg BID (1,500 mg/day), 500 mg three times a day (TID) (1,500 mg/day), 750 mg TID (2,250 mg/day), and 1,000 mg TID (3,000 mg/day). We have focused on the 30 New York subjects because complete clinical laboratory data are available for analysis and because these patients were, with a few exceptions (see “Missing and excluded data” below), kept on monotherapy for a full 56 days. Five subjects (one from the 500 mg BID group, 500 mg TID group, and one each from the 600 mg BID and 1,000 mg TID groups) were considered to have failed their initial regimen, and thus their data were not included in the analysis. The study used the same compassionate use protocol as the phase I/II studies of nelfinavir mesylate. Patients were seen at baseline and at 1, 2, 4, 7, 10, 14, 21, 28, 35, and 42 days of therapy.
TABLE 1. Reduction in HIV-1 RNA levels following potent combination therapy

| Interval (days) | log(M/M0) |
|----------------|-----------|
| 0–4            | 0.74      |
| 0–7            | 1.24      |
| 0–14           | 1.27      |
| 0–21           | 1.88      |
| 0–42           | 0.50      |
| 0–4–14         | 1.00      |
| 0–4–21         | 1.14      |

* Viral loads \( M \) and \( M_0 \) are defined by equation 5 of reference 26, with the death rate of short-lived infected cells (8) being 0.5 day\(^{-1} \), the death rate of long-lived infected cells (\( \alpha \)) being 0.04 day\(^{-1} \), and the proportion of virus that comes from the short-lived compartment (\( \eta \)) being 0.97. These values for \( \delta \), \( \mu \), and \( \eta \) are the averages of estimates for the five combination therapy patients in Table 2 of reference 26 and three combination therapy patients in Fig. 1 of reference 29. Reference 29 includes estimates for \( \mu \), \( \delta \), and \( \eta \) for additional patients, but these were obtained from a more complex model that incorporates data on infected cell populations. Since our formula for relative efficacy relies on plasma HIV-1 RNA data, we restricted ourselves to published estimates of \( \delta \), \( \mu \), and \( \eta \) obtained from fits to HIV-1 plasma RNA data alone.

The mean trough plasma drug concentration between visits. The date and time of the last nelfinavir dose prior to all outpatient visits was obtained and recorded. The mean trough plasma drug concentration on day 0 (hour 8 for TID patients and hour 12 for BID patients) and the predose measurements on days 7 and 14, while \( C_{0.14} \) was defined as the mean of first trough measurement on day 0 and the predose measurements on days 7, 14, and 21. For our statistical analyses, we scored drug dose in terms of the total number of milligrams of nelfinavir administered per day.

The concentration of nelfinavir in plasma was measured by high-pressure liquid chromatography at several times on day 0 and just before the first dose on days 7, 14, 21, and 28. Subjects were instructed not to take their scheduled morning dose of nelfinavir until after blood was drawn on the day of their clinic visits. The date and time of the last nelfinavir dose prior to all outpatient visits were obtained and recorded. The mean trough plasma drug concentration between days 0 and 14, \( C_{0.14} \), was defined as the mean of the first trough measurement on day 0 (hour 8 for TID patients and hour 12 for BID patients) and the predose measurements on days 7 and 14, while \( C_{0.21} \) was defined as the mean of first trough measurement on day 0 and the predose measurements on days 7, 14, and 21. For our statistical analyses, we scored drug dose in terms of the total number of milligrams of nelfinavir administered per day.

**Statistical measures of relative efficacy.** For each patient we calculated the relative efficacy of treatment using the formula

\[
\varepsilon_x = \frac{\log(V_0)}{\log(M/M_0)}
\]

where \( V_0 \) and \( V_0' \), respectively, refer to plasma HIV-1 RNA levels on days \( x \) and \( y \) following administration of the study drug (nelfinavir), while \( M_0 \) and \( M_0' \), respectively, refer to viral load on days \( x \) and \( y \) in studies of potent combination therapy (26, 29). \( M_0 \) and \( M_0' \) were quantified here using a mathematical model for the decline of plasma HIV-1 RNA levels following Pr inhibitor therapy (equation 5 in reference 26) with parameters set to means from studies of potent combination therapy in references 29 and 26 (see Table 1, footnote a, for details).

Relative efficacies less than 1.0 indicate treatments that reduce viral load at a lower rate than potent combination therapies, while relative efficacies greater than 1.0 indicate a rate of decline in virus concentration greater than the average observed during potent combination therapy. For efficacies based on analysis of more than two data points, the numerator of equation 1 was replaced by the slope of the regression line of through a graph of \( \ln(V) \) versus time and the denominator was replaced by \( \ln(M/M_0)V(V_0') \). Values for correlation coefficients involving relative efficacy are two-sided, except for forward regression tests, which are one-sided.

In the calculations that follow we start our analysis on day 4, i.e., with \( x \) being 4, because relative efficacies computed from day 4 onward bypass the shoulder phase of the response curve (i.e., the period of near steady-state viral load following the initiation of therapy) (32). The shoulder is shaped by factors, such as pharmacological and intracellular delays (14, 32), which are thought to have relatively little influence on the long-term rate of decline of virus. Another reason for using an \( x \) value of 4 is that we wish to correlate measures of treatment efficacy with viral load reduction at day 56, \( \log(V_0)/\log(V_0') \). Measures of relative efficacy based on the baseline viral load, \( V_0' \), may give spurious correlations because random variation in \( V_0' \) affects relative efficacy and viral load reduction at day 56 in the same way. Use of relative efficacies based on \( V_0' \) eliminates this statistical dependency. Another way to avoid this problem is to base the reduction in viral load at day 56 on a different, but related, measure of \( V_0' \), such as \( V_{1–7} \) (the value 7 days prior to the initiation of therapy). This method could not be used here, however, as some of our subjects discontinued their previous (non-Pr inhibitor) therapies only 2 weeks before entering the study.

**Missing and excluded data points.** For four subjects, samples were not obtained at day 56. For three of these subjects we used the next available data point (at days 64, 66, and 71) in place of the day 56 value, but the fourth was excluded from the analysis since a sample was not obtained from this subject again until day 91. Few patients, plasma HIV-1 RNA levels decreased very rapidly, falling below the level of detection (500 copies/ml) by day 14 or 21. Although relative efficacies based on the 500-copy/ml cutoff value for \( y \) (see equation 1 above) are minimal estimates, many of these estimates were higher than those obtained from patients in which \( y \) was not a cutoff value. We included these minimal estimates in the analysis if they were higher than the average relative efficacy for the study as a whole. This criterion for incorporating cutoff values allows us to include patients in which plasma HIV-1 RNA loads dropped from a high level to below the level of detection within the first 2 to 3 weeks.

Three subjects, all from the 600 mg BID group, discontinued therapy or failed to take drugs as scheduled (one discontinued therapy because of diarrhea at day 21, one was noncompliant from day 28 onward, and one had drug disruption at day 56). Data collected during and after these treatment interruptions were excluded from our analyses. Finally, for the five patients in which viral load had not fallen by 10-fold by day 28, we used day 28 (day 38 in one case) values in place of the day 56 value. This substitution is conservative because we have observed in several studies that once viral loads rebound in monotherapy patients they rarely decrease back to levels observed during the first 30 days of treatment in the absence of additional drug. This “ratcheting phenomenon” may be explained by evolution of drug-resistant genotypes as virus replicates in the presence of drug.

**RESULTS**

The mean reduction in viral load for the five treatment groups is presented in Fig. 1. Average viral load decreased rapidly in all treatment groups over the first 2 weeks of treatment. By day 56, viral load rebounded in four of the five treatment groups, with the largest rebounds occurring in the 1,000- and 1,200-mg/day treatment groups. In the 2,250-mg/day group (750 mg TID), plasma HIV-1 RNA levels fell below the limit of detection in four of the five patients by day 28; by day 56, however, viral load had rebounded in all but one of these patients. Plasma HIV-1 RNA levels in the 3,000-mg/day group showed more sustained declines, with viral load remaining at least 10-fold below the baseline level in five out of five subjects at day 56. The reduction in viral load by day 56, \( \log(V_0)/\log(V_0') \), was significantly greater in the 3,000-mg/day group than in the 2,250-mg/day group (Mann-Whitney U test, \( P < 0.05 \)).

Relative efficacies based on equation 1 for the five dosage groups are given in Table 2. To test the extent to which early measures of treatment efficacy based on only two viral load measurements predict viral load at later times, we regressed \( \varepsilon_x \) for various values of \( x \) and \( y \) against the logarithm of viral load reduction at day 56, \( \log(V_0)/\log(V_0') \) (using \( V_0' \) in place of \( V_0' \) for five patients as explained above). No correlation was ob-
served between viral load reduction at day 56 and measures of relative efficacy spanning the first 7 days of treatment, $e_{4,14}$, $e_{4,7}$, and $e_{4,0}$ (linear regressions: $R^2 = 0.002, R^2 = 0.105$, and $R^2 = 0.053$, respectively; none is statistically significant). This is consistent with the similar decays during the first 7 days seen in Fig. 1. Relative efficacies based on declines up to days 14 and 21 ($e_{4,14}$ and $e_{4,21}$), however, showed significant correlations with viral load reduction at day 56, with $e_{4,21}$ having a higher $R^2$ value than $e_{4,14}$ (Fig. 2). As discussed above, for this type of analysis, relative efficacies based on $x$ being 4 are preferable to those based on $x$ being 0 because log($V_{56}/V_0$) is not statistically independent of efficacies based on $V_0$.

The presence of points in the upper right regions of Fig. 2a and b shows that a high relative efficacy at weeks 2 and 3 is not always associated with a large viral load reduction at day 56. In the lowest-dosage group, for example, we obtained several early measures of relative efficacy above 1.0 in subjects whose viral loads later rebounded (data not shown). A low relative efficacy, by contrast, is rarely, if ever, associated with a good long-term response. Of the seven subjects with an efficacy, by contrast, is rarely, if ever, associated with a good long-term response. Of the seven subjects with an $e_{4,21}$ value below 0.5, for example, none had viral load reductions of 1 log or more at day 56 (Fig. 2b).

Relative efficacies based on linear regressions that include intermediate time points were similar to those found using the simple two-point method presented here (mean difference from two-point $e_{4,14}, 3.9\%$; mean difference from two-point $e_{4,21}, 8.0\%$). Correlations between relative efficacies calculated using linear regression over all points and reduction in viral load, log($V_{56}/V_0$), were also similar to those obtained using our two-point method (regression-based $e_{4,14}$, $R^2 = 0.36, P < 0.002$; regression-based $e_{4,21}$, $R^2 = 0.56, P < 0.001$). The similarity of the relative efficacies based on linear regressions to those based on our simple two-point method supports the use of this simpler and easier-to-use method.

The correlations in Fig. 2 include five patients for whom we used day 28 values (and one day 38 value) in place of day 56 values. These patients were dropped from the study because viral load was within 1 log of the baseline value at day 28. To verify that the correlations in Fig. 2 were not unduly influenced by these substitutions, we repeated these analyses without these patients. The corresponding $R^2$ values for this reduced data set were 0.27 and 0.47, respectively ($t$ tests on regression coefficients, $P < 0.02$ and $P < 0.002$, respectively), indicating that $e_{4,14}$ and $e_{4,21}$ continue to be correlated with the reduction in viral load at day 56 when these patients are removed from the analysis.

As expected, our measures of plasma drug concentration, $C_{0–14}$ and $C_{0–21}$, correlate with drug dose, with $C_{0–21}$ showing a slightly higher correlation with drug dose (Table 3). $C_{0–14}$ and $C_{0–21}$ also correlate with $e_{0,14}$ and $e_{0,21}$ (data not shown),

![FIG. 1. Mean reduction in HIV-1 RNA in plasma during the first 2 months for the five daily doses of nelfinavir.](image)

![FIG. 2. Correlation between reduction in HIV-1 RNA after 2 months of therapy, log($V_{56}/V_0$), and two measures of relative efficacy, $e_{4,14}$ and $e_{4,21}$. For the five subjects in which monotherapy was modified at day 28 due to a failure to achieve a 10-fold reduction in viral load, we used log($V_{56}/V_0$) in place of log($V_{28}/V_0$). As described in the text, we believe this substitution is conservative, because virus will usually continue to increase following a viral load rebound in patients who remain on Pr inhibitor monotherapy. Symbols are defined as in Fig. 1.](image)

**TABLE 2. Relative efficacy as a function of drug dose**

| Dose (mg/ml) | $e_{4,14}$ (x) | $e_{4,21}$ (x) |
|--------------|----------------|----------------|
| 1,000        | 0.95 ± 0.62 (5) | 0.14 ± 0.49 (3) |
| 1,200        | 0.64 ± 0.51 (5) | 0.69 ± 0.54 (4) |
| 1,500        | 0.82 ± 0.55 (10) | 0.70 ± 0.60 (10) |
| 2,250        | 1.09 ± 0.24 (4) | 0.84 ± 0.37 (4) |
| 3,000        | 1.42 ± 0.43 (5) | 1.23 ± 0.27 (5) |

* Values are means ± standard deviations; $n$ is the number of patients.
Since both plasma drug concentration and relative efficacy correlate with viral load reduction at day 56 individually, we also performed multiple regression analyses on log($V_{56}/V_0$) with plasma drug concentration and relative efficacy as independent variables. A forward stepwise regression analysis indicated that $e_{4,14}$ contributes only marginally to the regression sum of squares for viral load reduction at day 56 when the regression model already includes $C_{0,14}$ ($P$ for addition of $e_{4,14}$ to regression model, 0.057). However, a similar forward stepwise regression analysis using $e_{4,21}$ and $C_{0,21}$ reversed the order of importance: in this case $e_{4,21}$, but not $C_{0,21}$, contributed significantly to the regression sum of squares for viral load reduction at day 56 ($P$ for adding $C_{0,21}$, 0.025). In other words, relative efficacy appears to be a more important predictor of longer-term viral load reduction than plasma drug concentration when patients are monitored for 21 days. Of course, as shown in Fig. 2, in the absence of drug concentration data, both $e_{4,14}$ and $e_{4,21}$ are predictors of longer-term reductions in viral load.

The measure of relative efficacy introduced here is an empirical quantity that does not directly correspond to the antiretroviral efficacies considered in references 3, 9, 14, 31, and 40, in which the efficacy is a parameter in a mathematical model of HIV-1 dynamics. For an RT inhibitor the antiretroviral efficacy is defined in terms of the reduction in the infection rate constant, while for a PR inhibitor this efficacy is defined in terms of the reduction in the proportion of virions that are infectious. For dual-action combination therapy, the overall efficacy can be calculated in terms of these individual efficacy parameters (see references 40 and 31 for details). The empirical measure introduced in this paper, while lacking the mechanistic appeal of these mathematically motivated definitions, is a practical method for quantifying variation in the response to drug therapy.

### DISCUSSION

The development of a rapid and precise method for assessing antiretroviral efficacies of a novel antiretroviral compound in early clinical trials is highly desirable. We found that the relative efficacy, $e$, measured after only 14 days of treatment with nelfinavir correlates with overall reduction in viral load after 2 months, providing evidence for the predictive value of $e$ over short periods of time. We observed even greater correlations when relative efficacy was measured over 21 days of treatment, though we recognize that protocols this long may never be used due to concerns over the evolution of drug-resistant strains. We propose that the introduction of early measures of relative efficacy (i.e., of up to 14 days) into clinical phase I/II studies would allow for rapid assessment of antiviral activity of a particular dose of novel compounds and evaluation of dosage regimens. Relative efficacy should also be applicable to combination therapy regimens. Relative efficacy is a straightforward, easy-to-calculate alternative to the more complex multivariate methods previously presented by Mueller and colleagues (25).

Although early measures of relative efficacy correlate with later reductions in viral load, it is important to note that a high relative efficacy does not guarantee a good outcome. In Fig. 2a, for example, there is a high relative efficacy ($e_{4,14}$), 1.6 (small

### TABLE 3. Correlations between plasma drug concentration, dose, relative efficacy, and reduction in viral load by day 56a

| Measurements                  | $n$ | $R^2$ | $P$  |
|------------------------------|----|-------|-----|
| $C_{0,14}$ and dose           | 30 | 0.54  | <0.001 |
| $C_{0,21}$ and dose           | 29 | 0.59  | <0.001 |
| $e_{4,14}$ and dose           | 29 | 0.18  | <0.05 |
| $e_{4,21}$ and dose           | 26 | 0.25  | <0.01 |
| $e_{4,14}$ and $C_{0,14}$    | 29 | 0.31  | 0.002 |
| $e_{4,21}$ and $C_{0,14}$    | 26 | 0.40  | <0.001 |
| $C_{0,14}$ and $\log(V_5/V_0)$ | 26 | 0.40  | <0.001 |
| $C_{0,21}$ and $\log(V_5/V_0)$ | 26 | 0.37  | 0.001 |
| $e_{4,14}$ and $\log(V_5/V_0)$ | 25 | 0.36  | <0.002 |
| $e_{4,21}$ and $\log(V_5/V_0)$ | 23 | 0.61  | <0.001 |

a $C_{x,y}$, average trough concentration of drug in plasma between days $x$ and $y$; $n$, number of data points; $R^2$, square of the Pearson correlation coefficient.

### FIG. 3. Mean drug concentration in plasma during the first 4 weeks for each of the six dosage regimens. Error bars indicate 95% confidence intervals.
square, upper right), for a patient whose viral load had almost returned to baseline by day 56. By contrast, of the seven subjects with an $e_{421}$ of <0.5, none had a 10-fold reduction in viral load at day 56. We observed a similar pattern in a group of ritonavir monotherapy patients studied in reference 15, though the $R^2$ values were not statistically significant, as this study did not include as many patients (data not shown). For individual patients, therefore, relative efficacy appears to be better at predicting virological failure than at predicting success. This may be due to persistent or recurring problems that manifest themselves after therapy has been initiated, such as problems with adherence, changes in pharmacology, and the emergence of drug-resistant mutants.

Our finding that patients in 3,000-mg/day group had more sustained declines in plasma HIV-1 RNA levels than the 2,250-mg/day group should be interpreted cautiously. At higher doses, nelfinavir can lead to a number of adverse events, such as diarrhea and headache (21). The current recommendation of 2,250 to 2,500 mg/day (750 mg TID or 1,250 mg BID) strikes a balance between efficacy and toxicity and may still be the best choice for the majority of patients in clinical settings. In Agouron 511, a study in which nelfinavir was given in combination with zidovudine and lamivudine, viral load fell below the level of detection in a greater percentage of patients in the group receiving 2,250 mg of nelfinavir/day (750 mg TID) than in patients in the 1,500-mg/day group (500 mg TID). However, this study did not include a 3,000-mg/day group. Another finding that should be interpreted cautiously is our observation that plasma drug concentrations over the first 2 to 3 weeks of therapy were predictors for plasma viral load reduction at day 56. While plasma drug concentration predicted longer-term reductions in plasma HIV-1 RNA in this study, it may not have the same predictive power for other drugs. Some drugs may fail to fully penetrate anatomical or cellular sites of active viral replication, while others may have poor antiviral activity in vivo despite a high concentration in plasma. Relative efficacy, by contrast, is always of interest since it is a direct measure of the effect of drug on viral load. Relative efficacy has the further advantage of being based on a widely used and relatively routine measurement, plasma HIV-1 RNA level.

The fact that the earliest measures of relative efficacy (i.e., $e_{0.4}$, $e_{0.7}$, and $e_{4.2}$) did not correlate with drug dose suggests that the dosing regimens tested here may not differ very much in their ability to suppress susceptible or wild-type virus. The lack of variation in the earliest measures of relative efficacy suggests, contrary to the model proposed by Grossman et al. (11), that efficacies against sensitive virus are likely to be close to their upper limits (i.e., in the vicinity of 90% or greater). The divergence between the high- and low-dosage groups after day 14 and the apparent superiority of $e_{4.2}$ over $e_{4.14}$ with respect to predicting viral load reduction at day 56 could be due to the emergence of resistant or partially resistant genotypes between days 14 and 21. The finding by Markowitz et al. (21) of drug resistance genotypes after 90 days in 4 subjects in which virus rebounded is consistent with this hypothesis, but further investigation will be needed to prove that enough drug-resistant mutants were present in the low-dosage groups in the first 2 to 3 weeks to account for this divergence. Alternatively, subtle differences in the ability of the different dosage regimens to suppress sensitive virus may become more pronounced as the density of CD4$^+$ target cells increases, as one would predict from simple predator-prey models of HIV T-cell interactions (23, 27, 30, 40). Induction or down-modulation of host factors in response to declining HIV levels and increasing CD4$^+$ T-cell densities could also play a role. Further studies including quantitative measurements of HIV-1 RNA, plasma drug concentration, activated CD4$^+$ T cells (the putative target cells for HIV), and drug resistance during the first few weeks of treatment might help us distinguish between these competing hypotheses.

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