Viral Decay Dynamics in HIV-Infected Patients Receiving Ritonavir-Boosted Saquinavir and Efavirenz With or Without Enfuvirtide: A Randomized, Controlled Trial (HIV-NAT 012)

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The availability of enfuvirtide enables assessment of whether human immunodeficiency virus (HIV) decay can be enhanced by targeting reverse transcriptase, protease, and fusion. We performed a 12-week study of 22 patients randomized to receive ritonavir-boosted saquinavir and efavirenz with (the 3-target arm) or without (the 2-target arm) enfuvirtide. We observed no difference in the mean ± SD elimination-rate constant for overall decay (0.142 ± 0.040 per day and 0.128 ± 0.033 per day in the 2- and 3-target arms, respectively; P > .1) or for modeled first-phase decay rate (−0.62 ± 0.34 per day and −0.51 ± 0.16 per day; P > .1). Antiretroviral therapy that inhibits HIV reverse transcriptase and protease exerts potent antiviral effects that might not be augmented by the addition of an HIV fusion inhibitor.

The discovery that replication of HIV-1 in vivo occurs continuously at high rates and the availability of potent antiretroviral therapy (ART) has led to fundamental changes in treatment strategies for suppression of HIV replication [1]. It has been suggested that the viral suppression offered by a 3-drug antiretroviral regimen is inferior to that employing 5 drugs drawn from all 3 oral antiretroviral classes and that there is a relationship between pharmacokinetic exposure to antiretrovirals and the initial decline of plasma HIV RNA level [2, 3]. HIV RNA decay in plasma is biphasic: the initial rapid first-phase decay of plasma HIV RNA level is thought to be due to the loss of free virus and productively infected cells; the second, slower phase is thought to be due to the loss of long-lived infected cells [1, 4]. The more rapid the viral decline, the higher the efficacy of the antiviral therapy, with the effect being most apparent during the first phase [5, 6].

The availability of the fusion-inhibitor enfuvirtide affords an opportunity to reassess these dynamics to determine whether the addition of a third viral target (HIV fusion) to combination ART induces a more-potent viral decline. In 2002, the HIV Netherlands Australia Thailand Research Collaboration (HIV-NAT) conducted 3 pharmacokinetic research protocols that incorporated enfuvirtide [7, 8]. The study participants were guaranteed poststudy access to enfuvirtide as part of an effective ART regimen (enfuvirtide was provided by L. Hoffmann-La Roche, which has made enfuvirtide freely available lifelong to all patients who participate in pharmacokinetic studies with enfuvirtide, of whom a subset participated in the present study).

All eligible patients were offered entrance into a randomized trial primarily designed to examine the viral decay dynamics of antiviral therapy aimed at 2 versus 3 viral targets.

**Patients and methods.** Patient eligibility requirements were as follows: completion of 1 of 3 pharmacokinetic trials at HIV-NAT [7, 8]; a negative pregnancy test; use of barrier contraception; and use of enfuvirtide with other antiretrovirals. Exclusion criteria included the following: pregnant/breast-feeding; AIDS Clinical Trials Group grade 4 laboratory/clinical toxicity; alcohol/drug abuse; poor antiretroviral adherence in the past year; current severe illness/condition; or major organ allograft.

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The primary end point of the study was the comparison of the HIV elimination rate constant ($k$) for overall decay (days 2–28). Secondary end points were comparison of the estimate of $k$ for first-phase (days 2–8) and second-phase (days 8–28) responses as well as modeled first- and second-phase viral declines and the efavirenz C12h and saquinavir C_{min} pharmacokinetic levels at days 14 and 28. We estimated that 30 patients randomized equally to each arm would confer 80% power to detect a difference in estimated $k$ of 0.05 (2 tailed, $P < .05$). Patients attended clinic visits at baseline –7 days; baseline (day 0); days 2, 4, 6, 8, and 10; and weeks 2, 3, 4, 6, 8, 10, and 12. Participants were randomized 1:1 to receive either ritonavir-boosted saquinavir at 1000/200 mg twice daily and efavirenz at 600 mg nightly (the 2-target arm) or the same regimen with enfuvirtide at 90 mg subcutaneously twice daily (the 3-target arm); a random sequence was computer generated to maintain balanced arms.

Viral load was measured using the Amplicor HIV-1 Monitor Ultra Sensitive assay (Roche Diagnostics, F. Hoffmann-La Roche). To calculate the virion clearance rate constant, an exponential function was used to describe the rate of HIV RNA decline for each patient, as described elsewhere [2]. Viral load decay was analyzed using standard models of viral dynamics, as described elsewhere [4, 9, 10]. Differences in continuous variables between the 2 treatment arms were analyzed by use of Student’s $t$ test and the Mann-Whitney $U$ test. Statistical analyses were performed using SPSS (version 9.0; SPSS).

**Results.** Patients were well matched with regard to baseline HIV RNA level; the 3-target arm, compared with the 2-target arm, had a lower median body mass index (17.5 [range, 15.1–20.7] kg/m$^2$ and 22.5 [range, 17.3–27.6] kg/m$^2$, respectively) and a lower baseline median CD4 cell count (214 [range, 145–482] cells/mm$^3$ and 345 [range, 141–492] cells/mm$^3$, respectively). Patients were either ART naive or had been pretreated with nucleoside-analogue reverse-transcriptase inhibitors alone. All patients had previously received short courses of enfuvirtide as part of the pharmacokinetic trial protocols (total exposure to enfuvirtide ranged from 10 to 12 doses). Twelve patients were exposed to ritonavir monotherapy (200 mg twice daily) for a period of 3 days (6 doses) or ritonavir-boosted saquinavir (1000/100 mg twice daily) for 3 days (6 doses) [7, 8]. No patient had been previously exposed to nonnucleoside reverse-transcriptase inhibitors (NNRTIs). A minimum period of 13 months elapsed between the completion of all industry-sponsored pharmacokinetic studies and the screening visit for the current study. Viral load changes in individual patients and the mean changes in the 2 groups are shown in figure 1.

We found no significant difference for the estimated $k$ of overall viral decay between study arms. Estimates of the $k$ values in both arms of the study for 3 distinct periods (first phase, second phase, and overall viral decline) are shown in table 1.

For modeled decay estimates, we calculated from best-fit curves that the mean ± SD first-phase slope for the 2-target and 3-target arms were 1.03 ± 0.58 per day and 0.71 ± 0.27 per day, respectively ($P > .1$); for the second-phase slope, the results were 0.066 ± 0.038 per day and 0.039 ± 0.014 per day, respectively ($P = .05$). Restricting analysis to day 8 data, the best-fit curves for first-phase decay yielded average parameter values of −0.62 ± 0.34 per day and −0.51 ± 0.16 per day for the 2-target and 3-target arms, respectively ($P > .1$) (table 1).

For a model-independent assessment of the effect of enfuvirtide, we compared the drop in the viral load of each patient between days 2 and 8 (approximating the first-phase decline), between days 8 and 28 (approximating the second-phase decline), and between days 2 and 28 (overall decline). No significant differences were seen between the 2 arms in these comparisons (all $P > .1$; data not shown).

![Figure 1](image.png)

**Figure 1.** Viral load changes in individual patients (thin lines) and mean viral load changes (thick lines) in the 2-target (A) ($n = 11$) and the 3-target (B) ($n = 10$) arms.
Because we were unable to achieve our projected enrollment, we assessed the power of our study to detect differences in the viral decay rate by calculating 95% confidence intervals (CIs) around our estimate of the mean difference in overall decay constant between arms. For the primary end point, the mean difference in estimated viral decay constant between arms was 0.014 U, with a 95% CI lying between −0.019 and 0.048, thereby making differences of 0.05 or greater unlikely. The therapeutic ranges for both drugs were maintained well above the minimal accepted level throughout the period of first- and second-phase decay.

Discussion. Our results suggest that the addition of an antiretroviral agent aimed at inhibition of HIV fusion to an antiretroviral combination aimed at inhibition of the 2 conventional targets of reverse transcriptase and protease might not result in a more-potent viral decline than of that obtained by standard therapies targeting reverse transcriptase and protease alone in patients not previously heavily exposed to agents in these classes. There are a number of reasons why this might be the case. First, it is possible that in patients without resistance to HIV protease inhibitors (PIs) and NNRTIs adequately dosed therapy with these agents inhibits the 2 target viral enzymes so powerfully that the viral decay rate cannot be significantly augmented. The decay rates calculated for the ritonavir-boosted saquinavir and efavirenz arm in the present study are comparable with that calculated for a 4-drug combination regimen of ritonavir-boosted lopinavir at 533/133 mg twice daily, efavirenz at 600 mg once daily, tenofovir at 300 mg once daily, and lamivudine at 150 mg twice daily, as reported in a study by Louie et al. [5]. The regimen in the study by Louie et al. was estimated to be 1.3 times more potent with regard to first-phase viral decline when compared retrospectively with a cohort treated with another 4-drug combination of lamivudine, abacavir, and dual-PI therapy with indinavir and amprenavir and 1.47 times more potent than a regimen consisting of zidovudine, lamivudine, and dual-PI therapy with ritonavir and saquinavir. The prospective randomized nature of the current study enables a more-reliable comparison of relative declines between different regimens, and the addition of pharmacokinetic data demonstrating more than adequate levels of both saquinavir and efavirenz throughout the study period allows for confidence that optimal antiviral activity was exerted. It is possible that the improved potency with regard to first-phase decline in the comparator regimens in the study by Louie et al. might have been a function of inadequate PI pharmacokinetics and/or poor adherence to therapy rather than any intrinsic lack of antiviral potency between the compared regimens [11, 12]. The dual-PI regimens used in the comparator regimens in the study by Louie et al. are known to produce unpredictable PI pharmacokinetic levels and are often poorly tolerated [13]; as is the combination of ritonavir-boosted lopinavir and efavirenz [14]. Our data suggest that sufficient viral efficacy may be exerted by a simple combination of only 2 active drugs (saquinavir and efavirenz) and that the addition of an agent aimed at a novel target might not further augment potency to a measurable, and thereby clinically relevant, degree.

Recently, another group has published data on first-phase viral decay from a small, randomized study (n = 15 subjects) in which HIV-infected but ART-naive patients were randomized to receive a 4-drug regimen of ritonavir-boosted lopinavir, efavirenz, lamivudine, and tenofovir with or without the addition of enfuvirtide [15]. Interestingly, their assessment for first-phase decline calculated for the 4-drug (2-target) control group in their study was equivalent to the decay rate we calculated for our 2-target control group. However, in contrast to our results, they demonstrated a 22% increase in the rate of first-phase decay measured between days 1 and 6 for the enfuvirtide-containing arm, compared with that in the non–enfuvirtide-containing control arm. Close inspection of their study suggests, however, that the (statistically significant) difference between the study arms appears to be accounted for by a more-pronounced viral load increase in the enfuvirtide-containing arm than in the comparator arm after initiation of ART but before commencement of the first-phase decline, because there was no statistically significant difference in viral loads between the study and control groups at days 0 and 6. The phenomenon of transient viral load increases immediately after initiation of therapy has been observed and reported elsewhere [4].

Table 1. Estimated elimination rate constants (k) for viral decay (overall, first-phase, and second-phase) and estimated first-phase HIV-RNA decay for 2-target therapy versus 3-target therapy.

<table>
<thead>
<tr>
<th>Category</th>
<th>2-target arm (n = 11)</th>
<th>3-target arm (n = 10)*</th>
<th>P</th>
<th>Difference between study arms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall k/day</td>
<td>0.142 (0.115 to 0.169)</td>
<td>0.128 (0.104 to 0.152)</td>
<td>&gt;.1</td>
<td>0.014 (–0.019 to 0.048)</td>
</tr>
<tr>
<td>First-phase k/day</td>
<td>0.449 (0.406 to 0.492)</td>
<td>0.441 (0.380 to 0.502)</td>
<td>&gt;.1</td>
<td>0.008 (–0.062 to 0.078)</td>
</tr>
<tr>
<td>Second-phase k/day</td>
<td>0.082 (0.049 to 0.115)</td>
<td>0.072 (0.044 to 0.100)</td>
<td>&gt;.1</td>
<td>0.010 (–0.031 to 0.051)</td>
</tr>
<tr>
<td>First-phase HIV RNA decay/day</td>
<td>−0.62 (–0.848 to 0.392)</td>
<td>−0.51 (–0.647 to 0.403)</td>
<td>&gt;.1</td>
<td>0.109 (–0.123 to 0.341)</td>
</tr>
</tbody>
</table>

NOTE. Data are estimated means (95% confidence intervals). 2-target arm, saquinavir, ritonavir, and efavirenz; 3-target arm, saquinavir, ritonavir, and enfuvirtide.

* Eleven patients were originally enrolled, but 1 patient dropped out of the study at day 10.
Our calculations had predicted that we would require 30 patients to find a difference in $k$ of 0.05. We were, however, only able to recruit 22 of the potential 36 patients into the study. It is, therefore, possible that the present study was underpowered to find a small but significant difference in potency between the 2 regimens. However, against expectations, our results slightly favored the 2-target arm. A calculation of the 95% CIs around our estimate of the mean difference in overall decay constant between arms demonstrated that this would lie at a value between $-0.019$ and $0.048$. This indicates that differences larger than 0.05 (the value that we originally powered the study to detect) are inconsistent with our data and therefore unlikely.

An alternative explanation for the lack of increased potency in the 3-target arm might be that patients could have developed resistance to the fusion inhibitor and/or to saquinavir as a consequence of the brief exposure to these agents during the conduct of the prior pharmacokinetic studies. We cannot exclude this possibility, although exposure during the pharmacokinetic studies was minimal; all subjects received between 10 and 12 doses of enfuvirtide and were exposed to either ritonavir monotherapy or ritonavir-boosted saquinavir for 6 doses only, making selection of resistance unlikely [7, 8]. In any event, if enfuvirtide mutations had been selected, current evidence suggests that enfuvirtide mutations disappear fairly promptly after withdrawal of the drug [16] and that, given the 13-month delay between completion of the pharmacokinetic studies (and cessation of enfuvirtide) and the commencement of the current study, it is unlikely that the early viral declines would be affected by any archived enfuvirtide resistance mutations. In addition, the patients exposed to ritonavir-boosted saquinavir were distributed evenly between arms (data not shown), and it is therefore unlikely that any PI resistance mutations would have had a bearing on the integrity of the comparative result.

In conclusion, this randomized study suggests that relatively simple antiretroviral combination regimens, when used at maximal efficiency, may exert sufficient potency for the optimal treatment of chronic HIV infection. The issue is worthy of further investigation.

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References


