

# A randomized pilot study comparing combination therapy plus enfuvirtide versus a treatment interruption followed by combination therapy plus enfuvirtide

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Most individuals with multidrug-resistant HIV who switch to a new therapeutic regimen containing a single fully effective agent experience incomplete viral suppression. We postulated that interruption of antiretroviral therapy prior to the introduction of such a regimen would improve long-term virological outcomes. Thirty, three-class experienced, enfuvirtide-naïve individuals with detectable drug-resistant viraemia were randomized to an immediate enfuvirtide/optimized-background treatment regimen or a 16-week treatment interruption followed by enfuvirtide/optimized-background treatment regimen. The median CD4<sup>+</sup> T-cell count and viral load at study entry were 39 cells/mm and 4.72 log<sub>10</sub> copies

RNA/ml, respectively. There was no evidence of any virological or immunological benefit associated with the interruption. In multivariate analysis, only the baseline phenotypic susceptibility score was predictive of treatment response at week 48 ( $P=0.009$ ). Only 40% of individuals had evidence of a shift in drug-resistance genotype during the interruption. In summary, interrupting therapy prior to initiating salvage therapy in patients with advanced disease did not result in an improved virological response to enfuvirtide. The collective predictive activity of an enfuvirtide-containing regimen was important in predicting treatment response.

## Introduction

The optimal therapeutic approach for the more heavily treatment-experienced patient with drug-resistant viraemia has not been established [1,2]. This is particularly true for heavily pre-treated individuals with only one therapeutically effective agent remaining. Historically, such individuals often experience a transient virological response to subsequent therapy [3,4]. For these reasons, individuals with a limited number of therapeutic options remaining are often maintained on a stable, well-tolerated regimen, even as ongoing viral replication is evident. Although continuing a partially suppressive regimen may be appropriate for some patients, many patients with multidrug-resistant virus continue to immunologically progress and, therefore, need to modify therapy even if the number of fully effective agents is limited [5].

Interrupting antiretroviral therapy after the emergence of drug-resistant HIV-1 is often associated with a shift in the dominant plasma virus population from a drug-resistant to a drug-susceptible population [6–10]. These observations led to the hypothesis that interrupting

therapy prior to a new salvage regimen may be clinically useful [8,11–13]. In a prior study of 24 individuals interrupting therapy, a transient virological response was observed in patients who initiated a regimen containing no drug to which their pre-interruption virus was fully susceptible. In contrast, durable viral suppression (viral load <200 copies/ml through week 72) was observed in all patients who initiated a regimen containing only one drug to which their pre-treatment virus was fully susceptible [14]. This occurred despite moderate-level to high-level phenotypic resistance to other drugs in the treatment regimen, and contrasts with other data suggesting that combination therapy should include at least two or preferably three active agents. Based on these observations, we hypothesized that treatment interruptions may be most beneficial in a carefully selected cohort of individuals whose virus was resistant to most available drugs, but may yet be sensitive to a new therapeutic class. This hypothesis was tested in a pilot study of 30, three-class experienced individuals who were enfuvirtide-naïve and had to switch to an enfuvirtide-based regimen.

## Methods

### Study design

This is a single-centre, open-label, randomized study. Eligibility criteria included detectable viraemia (plasma HIV RNA levels >500 copies/ml) on a stable combination antiretroviral regimen for at least 24 weeks and documentation of recent plasma HIV RNA levels >500 copies RNA/ml. Individuals must also have had a screening phenotypic/genotypic resistance assay demonstrating resistance to nucleoside analogues, non-nucleoside reverse transcriptase inhibitors and protease inhibitors (PIs).

The study investigators and the individual's primary healthcare provider constructed the optimized background regimen prior to randomization. Several factors, including treatment history and resistance testing, were used to construct this regimen. Whenever possible, individuals received a combination regimen that included at least one ritonavir-boosted PI and at least two nucleoside analogues. Enfuvirtide was administered twice daily at 90 mg.

After determining the study regimen, individuals were randomized to either immediate therapy with enfuvirtide/optimized-background regimen or a 16-week treatment interruption followed by an enfuvirtide/optimized-background regimen. Individuals undergoing a treatment interruption were encouraged to resume therapy at or after week 8 if plasma HIV RNA levels increase by more than one  $\log_{10}$  copies/ml, or if CD4<sup>+</sup> T-cell counts decreased by more than 50%. Week 8 was felt to be a sufficiently long enough period of observation off drugs given the benefit of this approach in an earlier randomized treatment interruption study (ANRS 097) [15].

### Measurements

Phenotypic susceptibility was measured at baseline using the PhenoSense assay (Monogram Biosciences Inc, San Francisco, CA, USA). For analytical purposes, phenotypic susceptibility was defined based on lower and upper clinical 'cut-offs', which have been estimated for the interpretation of this assay (personal communication, Michael Bates, Monogram Biosciences, Inc). The phenotypic susceptibility score (PSS) was calculated as follows: if the drug was fully active (fold-change in 50% inhibition concentration [ $IC_{50}$ ] below the lower cut-off), a score of one was used; if the drug was partially effective (fold change in  $IC_{50}$  between the lower and upper cut-off), a score of 0.5 was used; and if the drug had no clear activity (fold change in  $IC_{50}$  above the upper fold change), a score of 0 was used.

Enfuvirtide drug susceptibility and viral tropism were measured using single-cycle replication-entry assays, in which patient-derived *pol* genes were

co-transfected with a luciferase-containing, *env*-defective HIV genomic vector. Infectivity was assessed by measuring the production of relative light units in cell lines expressing CCR5 or CXCR4.

### Statistical analysis

The primary endpoint for the randomized clinical study was the proportion of individuals with plasma HIV RNA levels below the level of quantification (<75 copies RNA/ml) 48 weeks after initiating an enfuvirtide-based regimen. An isolated episode of detectable viraemia (>75 copies RNA/ml) at week 48 was not considered as evidence of virological failure if the preceding and subsequent HIV RNA levels were undetectable. Individuals who interrupted therapy prior to week 48 were considered as 'failures' if they exhibited evidence of incomplete viral suppression, as defined by the U.S. Department of Health and Human Services treatment guidelines (for example, confirmed HIV RNA level >400 copies/ml after 24 weeks or a repeated HIV RNA level >400 copies/ml after prior suppression of viraemia to <400 copies/ml) [16]. Individuals who discontinued therapy due to intolerance prior to study week 48 were considered a success if they did not meet these definitions of virological failure at the time therapy was interrupted.

A sample size of 30 (15 per arm) was chosen based on the assumption that those in the control arm would have a response rate comparable to those observed in the TORO studies (that is, an approximately 30% durable virological response rate) [3,4]. Moreover, we assumed that the response to a regimen containing enfuvirtide as the only fully effective agent would be comparable to that observed in our previous treatment interruption study (that is, an approximately 70% durable virological response rate) [14]. Assuming no patients were lost to follow-up, then a sample size of 30 provided an 80% power to detect such a treatment response.

## Results

Thirty individuals were randomized to an immediate enfuvirtide-based regimen versus a structured treatment interruption followed by an enfuvirtide-based regimen. The median baseline CD4<sup>+</sup> T-cell count was 39 cells/mm<sup>3</sup> (interquartile range [IQR] 12–135) and baseline HIV RNA level was 4.72  $\log_{10}$  copies RNA/ml (Table 1). The median fold change in  $IC_{50}$  for abacavir was 5.5, which indicates moderate to high level resistance for this therapeutic drug class (abacavir susceptibility was used as a surrogate for nucleoside reverse transcriptase inhibitor resistance because abacavir resistance is strongly correlated with resistance to other drugs and because abacavir resistance from most individuals varies over the dynamic range of the assay

**Table 1.** Baseline characteristics

Characteristic	Immediate	Interruption	All
HIV RNA, log	4.65 (4.48–5.11)	4.72 (4.45–5.07)	4.72 (4.43–5.14)
CD4 <sup>+</sup> T cells, cells/mm <sup>3</sup>	26 (7–139)	47 (13–94)	39 (12–135)
Abacavir, fold-change IC <sub>50</sub>	6.8 (4.0–9.0)	4.6 (3.0–8.2)	5.5 (3.2–8.5)
Lopinavir, fold-change IC <sub>50</sub>	41 (1.4–124)	69 (5.3–179)	51 (2.6–171)
Enfuvirtide, IC <sub>50</sub>	0.021 (0.015–0.032)	0.037 (0.030–0.049)	0.031 (0.016–0.043)

Baseline was defined as the date of randomization. All values reflect median and interquartile ranges. IC<sub>50</sub>, 50% inhibition concentration

and because abacavir resistance is not reduced by M184V) [17]. The median fold-change in IC<sub>50</sub> for lopinavir was 51.

The median duration of treatment interruption was 15.9 (IQR 11.5–16) weeks. During the treatment interruption, CD4<sup>+</sup> T-cell counts decreased by a median of 27 cells/mm<sup>3</sup> (IQR –58 to –6) and HIV RNA increased by 0.42 (IQR 0.13–0.56) log copies/ml.

#### Virological and immunological response to enfuvirtide therapy

Three individuals discontinued enfuvirtide due to intolerance; one prior to week 2 (this individual’s data were not included in subsequent analyses). One individual developed bacterial pneumonia and died 4 weeks after initiating enfuvirtide. Ten individuals with confirmed virological failure of their enfuvirtide-based regimen discontinued enfuvirtide at or after 24 weeks of treatment.

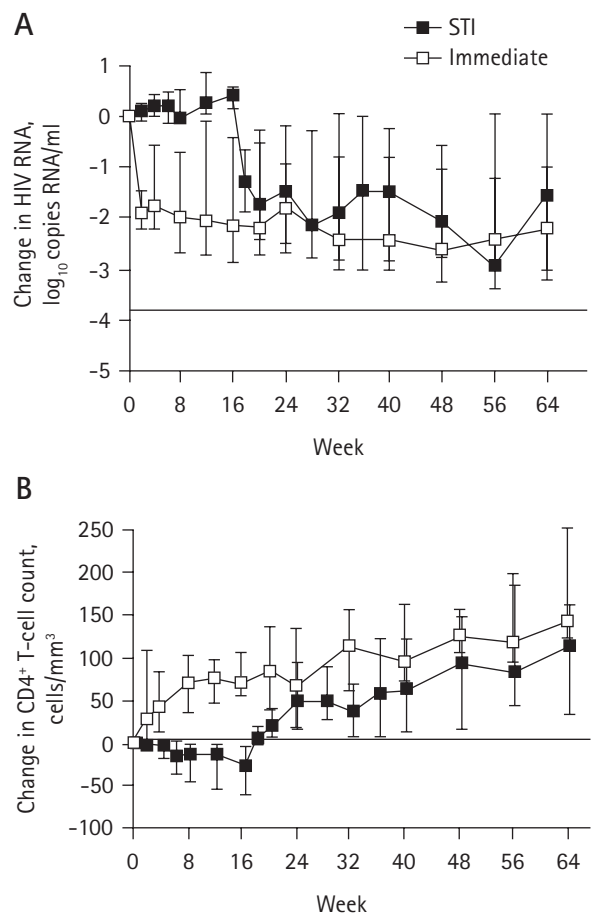
Using a last-value carried forward analysis, there was no difference in virological outcomes at week 48; 8 of 15 (53%) individuals in the immediate therapy group whereas 5 of 14 (36%) of patients in the treatment interruption group had a viral load <75 copies/ml (*P*=NS). The median change in plasma HIV RNA levels from study baseline (date of randomization) to week 24 of enfuvirtide was –1.82 log<sub>10</sub> copies/ml (IQR –2.65 to –0.28) in the immediate arm and –1.38 log<sub>10</sub> copies/ml (IQR –3.21 to –0.47) in the interruption arm (Figure 1A); these trends persisted through week 48 (although many individuals with detectable viraemia stopped enfuvirtide before week 48). The median change in CD4<sup>+</sup> T-cell counts at week 24 of enfuvirtide was 69 cells/mm<sup>3</sup> (IQR 31–133) in the immediate arm and 69 cells/mm<sup>3</sup> (IQR 39–177) in the interruption arm (*P*=NS; Figure 1B).

#### Predictors of virological response to enfuvirtide-based therapy

The collective predictive activity of an enfuvirtide-containing regimen (as defined by the phenotypic susceptibility score), but not enfuvirtide baseline susceptibility was important in predicting treatment

response. All individuals with a phenotypic susceptibility score of ≤1 failed to achieve a durable virological response; in contrast, 63% of individuals with a score of >1 achieved had an undetectable viral load through to week 24. In univariate models, the baseline phenotypic

**Figure 1.** Treatment outcomes in subjects interrupting versus not interrupting therapy prior to enfuvirtide



The change in HIV RNA levels and change in CD4<sup>+</sup> T-cell counts are presented for patients randomized to either immediate enfuvirtide-based regimen or an interruption followed by an enfuvirtide-based regimen. Baseline refers to the date of randomization; most individuals were randomized to an interruption resumed therapy at week 16. There no difference in virological outcomes 24 or 48 weeks after the resumption of therapy. STI, structured treatment interruption.

susceptibility score was the only factor predictive of virological success at week 48. For every additional active drug there was a threefold increase in the odds of achieving an undetectable viral load through to week 48 ( $P=0.009$ ). Treatment arm, baseline viral load, baseline CD4<sup>+</sup> T-cell count, co-receptor tropism or baseline enfuvirtide susceptibility ( $IC_{50}$ ) did not predict outcome.

#### Factors associated with loss of drug resistance during a treatment interruption

Only 40% of individuals randomized to a treatment interruption showed complete or partial genotypic reversion to wild-type during the interruption period. This rate of losing genotypic resistance was much lower than that observed in our prior study [7]. In an analysis of our 15 patients who interrupted therapy in this study, no single factor was consistently predictive of resistance changes during the interruption period. However, a lower baseline CD4<sup>+</sup> T-cell count was associated with a twofold decrease in probability of not exhibiting a shift in resistance during the interruption phase ( $P=0.20$ ). When we included individuals from this and our prior study in a single post-hoc analysis ( $n=38$ ), a lower pre-interruption CD4<sup>+</sup> T-cell count was associated with a lower probability of a shift in drug-susceptibility ( $P=0.01$ ), after adjusting for all other factors including plasma HIV RNA levels, pre-interruption replicative capacity, fold-change in  $IC_{50}$  to abacavir and fold-change in  $IC_{50}$  to the administered protease inhibitor.

## Discussion

The role of treatment interruptions in patients with multidrug-resistant HIV has been extensively studied. At least four randomized clinical trials demonstrated no evidence of a virological, immunological or clinical benefit of a structured treatment interruption among individuals with multidrug-resistant HIV [8,11–13]. In our current study, we focused on a specific subset of individuals with drug-resistant HIV: those with three-class resistance and who were initiating a new salvage regimen containing a new therapeutic class. We observed no evidence of a therapeutic benefit in interrupting therapy prior to initiation of a new ‘salvage’ regimen. Hence, our study is generally consistent with current clinical guidelines and does not support the use of structured treatment interruptions as a strategy in patients with multidrug-resistant HIV.

This study has several limitations which deserve comment. First, this study was powered based on the assumption that the vast majority of individuals in the control arm would have an incomplete response to therapy. Our response rate in this arm was greater than

expected, perhaps because of a higher than expected use of partially effective agents. Although it is possible that a larger study in this patient population may have demonstrated benefit, the lack of any positive trends here makes this unlikely. Second, this study was based on the assumption that all individuals in the interruption arm would experience a shift in dominant virus population from resistance to susceptibility. The degree to which this occurred was much lower than expected, perhaps due to the advanced nature of our population. Theoretically, advanced immunodeficiency may be associated with a virus population with preserved replicative capacity and/or a limited persistence of wild-type HIV in a latent reservoir; both of these factors could result in persistence of drug-resistance during an interruption.

In conclusion, we found no benefit of a treatment interruption for triple-class experienced individuals initiating a regimen containing a novel therapeutic drug class. Our data, including the data of several others studies, suggest that the best management of individuals who have a progressive disease in face of drug-resistant HIV is to modify therapy using a regimen containing more than one effective drug. Even in patients with no effective ‘background’ drugs, measurable immunological benefit can be achieved with enfuvirtide, thus suggesting that this drug should be used in patients with very advanced disease and limited therapeutic options.

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