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HIV-1-infected patients from the French National Observatory experiencing virological failure while receiving enfuvirtide

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***Objectives*: We studied *gp41* mutations associated with failing enfuvirtide salvage therapy.**

***Methods*: This multicentre study involved patients with HIV-1 plasma viral load (pVL) > 5000 copies/mL after at least 3 months of uninterrupted enfuvirtide therapy and with plasma samples available at inclusion (T0), at initial enfuvirtide failure (T1) and at last follow-up visit during continued failing enfuvirtide therapy (T2). The HR-1 and HR-2 domains of the *gp41* gene were sequenced at T0, T1 and T2.**

***Results*: Ninety-nine patients were enrolled. At baseline, the median pVL and CD4 cell count were 5.1 log copies/mL and 72 cells/mm^3, respectively. Based on the ANRS Resistance Group algorithm, the proportion of patients harbouring viruses with enfuvirtide resistance mutations increased significantly between T0 and T1. In the HR-1 domain, the V38A/M, Q40H, N42T, N43D and L45M mutations were**

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selected ($P < 0.02$). In the HR-2 domain, no mutations were significantly selected during the follow-up. None of the mutations was associated with a CD4 cell count increment.

***Conclusions*: Mutations selected during failing enfuvirtide salvage therapy are mainly located in the HR-1 domain of the *gp41* gene, between codons 38 and 45. No mutations were associated with an increase in the CD4 cell count.**

Keywords: HIV drug resistance, resistance mutations, *gp41*, T-20

Introduction

Enfuvirtide-resistant viral populations have been found to bear mutations in the highly conserved three amino acid motif at positions 36–45 of the HR-1 domain of the *gp41* gene. Other studies have also shown mutations in a wider region of HR-1 (amino acids 36–45). The aim of this study was to identify mutations in the HR-1 and HR-2 domains of the HIV-1 gp41 protein in heavily treated patients in whom enfuvirtide-containing salvage therapy was initially effective and then failed.

Patients and methods

This multicentre study involved 99 heavily treated enfuvirtide-naive HIV-1-infected patients from 15 HIV/AIDS centres. They all experienced virological failure while receiving an enfuvirtide-containing salvage regimen with plasma viral load >5000 copies/mL after at least 3 months on enfuvirtide combination therapy. Plasma samples were available at baseline (T0), at the time of virological failure (T1) and, in 55 cases, during continued failing enfuvirtide therapy (T2). All patients gave written informed consent, and the study was approved by the Comité Consultatif de Traitement de l'Information dans la Recherche Scientifique et Médicale and the Commission Nationale Informatique et Libertés.

Population-based sequencing of the HR-1 and HR-2 domains of the *gp41* gene was performed at T0, T1 and T2 by using the ANRS protocol (www.hivfrenchresistance.org). Enfuvirtide mutations were identified from the IAS-USA 2006 expert list of mutations.[1] Genotypic susceptibility to enfuvirtide was predicted with the 2007 ANRS resistance rules (www.hivfrenchresistance.org), and resistance to a class was defined as resistance to at least one member of the class.

The MacNemar test was used to compare the frequency of mutations between T0, T1 and T2. Changes in the CD4 cell count were compared between patients with and without specific mutations by using the non-parametric Mann–Whitney test. Changes in continuous variables were tested with the paired Wilcoxon test.

Results

Baseline characteristics of the 99 patients enrolled in the study are summarized in Table 1. The median number of drugs predicted to be effective, in addition to enfuvirtide, was 1

Table 1. Patients' baseline characteristics

Characteristic[a]	All patients ($n = 99$)	Patients failing enfuvirtide ($n = 55$)[b]
Male	88 (89%)	48 (87%)
Transmission group		
homosexual	50 (51%)	21 (38%)
heterosexual	26 (26%)	19 (35%)
IV drug users	12 (12%)	9 (16%)
unknown	11 (11%)	6 (11%)
HIV-1 subtype B	85 (86%)	48 (87%)
Age	43 (38–49)	45 (37–51)
Duration of seropositivity (years)	12 (9–16)	12 (9–16)
Duration of antiretroviral therapy (years)	9 (8–11)	9 (8–11)
Number of previous antiretroviral drugs		
PI	4 (4–5)	5 (5–7)
NRTI	6 (5–7)	6 (5–7)
NNRTI	1 (1–2)	1 (1–2)
HIV-1 plasma RNA ($\log_{10}$ copies/mL)	5.1 (4.6–6.6)	5.0 (4.5–5.5)
HIV-1 plasma RNA zenith ($\log_{10}$ copies/mL)	5.7 (5.3–5.9)	5.7 (5.4–5.9)
CD4 cell counts (cells/mm^3)	72 (13–150)	83 (17–169)
Nadir CD4 count (cells/mm^3)	16 (13–19)	15 (13–18)
Time from baseline to virological failure (weeks)	16 (13–19)	15 (13–18)

[a]Values are either number (percentage) or median (interquartile range).
[b]Patients with at least two samples during enfuvirtide failure.

(range 0–5); 12, 46, 28 and 13 patients had viruses with resistance to zero, one, two and three classes of antiretroviral drugs, respectively. At baseline, four patients had viruses with a single enfuvirtide resistance-associated mutation (V38A, V38E, Q40Q/H and N42T) and two patients had viruses with two such mutations (G36S+N43S and Q39R+N42S). Five positions (42, 46, 54, 72 and 77) were polymorphic (>10%) in the HR-1 domain. In contrast, extensive polymorphism was observed in 12 positions (119, 121, 122, 125, 130, 135, 137, 147, 148, 151, 157 and 160) within the HR-2 domain.

Baseline characteristics of the 55 patients remaining on enfuvirtide therapy despite virological failure were similar to those of the whole population. Three of them had viruses bearing mutations (V38A, Q40Q/H and N42T). Eight (15%), 21 (38%), 17 (31%) and 9 (16%) of these patients had viruses with resistance to zero, one, two and three classes of antiretroviral drugs, respectively.

The enfuvirtide resistance mutations selected between baseline and virological failure are shown in Figure 1(a) for the 99 patients and in Figure 1(b) for the subgroup of 55 patients. Six mutations (V38A/M, Q40H, N42T, N43D and L45M) were selected in the HR-1 domain (all $P < 0.02$, MacNemar test). In the subgroup of 55 patients, mutations G36D, Q40H, N42T, N43D and L45M were selected between baseline and the

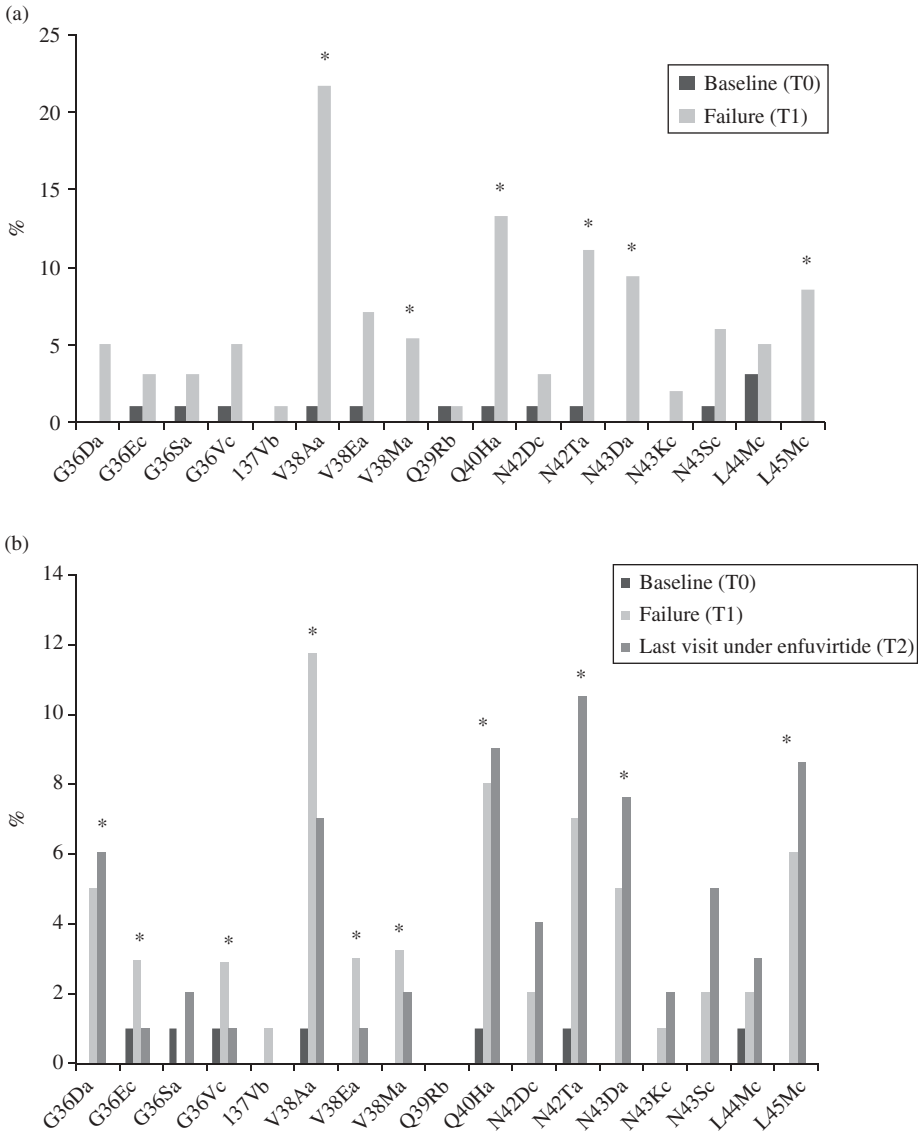

Figure 1. Selection of mutations in the *gp41* HR-1 domain. (a) Selection of mutations in the HR-1 domain of *gp41* between baseline (T0) and initial virological failure (T1) ($n = 99$). (b) Selection of mutations in the HR-1 domain of *gp41* between baseline (T0), initial virological failure (T1) and last follow-up visit under enfuvirtide (T2) ($n = 55$). Lowercase letters added to amino acid changes are as follows: a, mutations associated with enfuvirtide resistance in both the IAS-USA 2006 and ANRS 2007 lists; b, mutations associated with enfuvirtide resistance in the IAS-USA 2006 list only; c, mutations associated with enfuvirtide resistance in the ANRS 2007 list only. Asterisks in (a) indicate enfuvirtide resistance mutations significantly selected between baseline (T0) and initial virological failure (T1) ($P < 0.05$). Asterisks in (b) indicate enfuvirtide resistance mutations significantly selected or deselected between baseline (T0) and initial virological failure (T1) or the last follow-up visit on enfuvirtide (T2) ($P < 0.05$).

follow-up sample on failing enfuvirtide therapy (all $P < 0.02$). Mutations V38A/E/M reverted to the wild-type allele during continued failing enfuvirtide therapy ($P = 0.02$).

At initial virological failure, the median number of enfuvirtide resistance mutations was 1 (IQR: 0–1). The proportion of patients with at least one enfuvirtide resistance mutation in the *gp41* gene was 71% ($n = 70$) in the whole population of 99 patients and 73% ($n = 40$) in the subgroup of 55 patients. The corresponding rates were 75% ($n = 74$) and 76% ($n = 42$) based on the ANRS 2007 resistance rules. No particular mutations in the *gp41* HR-2 domain were significantly selected between baseline and initial virological failure or between baseline and the final sample during continued failing enfuvirtide therapy, although R122K and S138A tended to be selected in the HR-2 domain between baseline and continued failing enfuvirtide therapy ($P = 0.063$).

The median change in the HIV-1 viral load between baseline and initial virological failure was -0.2 log copies/mL (IQR: -0.5 to -0.2) ($P < 0.001$). A median gain of 27 CD4 cells/mm^3 (IQR: 0–82) was observed between baseline and virological failure ($P < 0.001$). In the subgroup of 55 patients, no significant change in the HIV-1 plasma RNA or in the CD4 cell count was observed between initial virological failure and the follow-up sample during continued enfuvirtide therapy.

None of the mutations selected in the HR-1 domain at initial virological failure was associated with the change in the CD4 cell count between baseline and initial virological failure. However, between initial failure and the follow-up sample, the presence of one or more enfuvirtide resistance mutations was associated with a significant loss of CD4 cells (-29 cells/mm^3; $P = 0.039$). In particular, mutations V38A and N42T had a negative impact on the CD4 cell count, with significant losses of 39 and 32 cells/mm^3, respectively ($P = 0.05$). Moreover, mutation V38M was associated with a significant increase in the median HIV-1 viral load ($+0.4$ log copies/mL). Interestingly, patients with mutation G36D had an increase of 26 CD4 cells/mm^3, whereas patients with G36 had a decline of 27 CD4 cells/mm^3, but the difference was not significant ($P = 0.14$).

Discussion

In one of the largest observational studies of enfuvirtide salvage therapy, we have confirmed reports that amino acids 36–45 of the HR1 domain are the main determinants of resistance to enfuvirtide, as mutations in this region emerged in more than 70% of our patients 3 months after enfuvirtide salvage therapy had started to fail.

Interestingly, some patients had baseline mutations in the HR-1 domain, despite being enfuvirtide naive prior to inclusion. This has already been described in other studies.[2,3]

Mutations V38A/M, Q40H, N42T, N43D and L45M in the HR-1 domain were significantly selected between baseline and initial virological failure. The N42D mutation, listed in the ANRS algorithm, was described in patients failing enfuvirtide therapy and shown to confer decreased sensitivity to enfuvirtide when present in site-directed mutants or in recombinant viruses. In contrast, this mutation has not been noted in clinical isolates with naturally occurring *gp41* HR1 variations described in enfuvirtide-naive patients.[3,4] In our study, the N42D mutation was selected between baseline and virological failure, but this was not statistically significant. This raised the question of whether to maintain this mutation in algorithms designed to interpret enfuvirtide resistance.

Mutations V38A/E/M were most frequently selected (36%), as reported previously.[5] Cabrera *et al.*[6] studied a small number of heavily treated patients and found that mutations at positions 36 and 38 emerged rapidly (median 10 weeks) upon failure but subsequently disappeared in most patients. We also found that mutations V38A/E/M reverted to the wild type when the failing enfuvirtide regimen was pursued, whereas mutations at position 36 did not revert, contrary to previous findings. In the study by Cabrera *et al.*,[6] the HR-1 domain was sequenced at weeks 4, 12, 24, 48 and 96, whereas we sequenced HR-1 only at baseline, at initial virological failure and, in some patients, during failing enfuvirtide therapy.

The *gp41* HR2 domain was more polymorphic than HR1, as reported elsewhere.[5,7] Some mutations in HR-2 have been suggested to contribute to enfuvirtide resistance,[8,9] but no specific HR-2 mutations emerged during our study. Further studies are required to determine whether these mutations in the HR-2 domain are involved in resistance to enfuvirtide.

Unlike Aquaro *et al.*,[5] we observed no specific combinations of mutations affecting both the HR1 and HR2 domains during enfuvirtide treatment. Our findings do not confirm that the S138A mutation in HR2 compensates for mutations at position 43.[6]

Contrary to Svicher *et al.*,[10] we found that no specific patterns of mutations in the HR-1 domain (e.g. V38A/E, Q40H + L45M, V38A + T18A, V38A+N140I or V38A + T18A + N140I) were associated with a CD4 cell count increment during the period of our study. Mutation G36D was associated with an increase of 26 CD4 cells/mm^3, compared with a decrease of 27 cells/mm^3 in patients with the wild-type allele, but the difference was not statistically significant ($P = 0.14$), and it is difficult to differentiate between a lack of statistical power and a true phenomenon as this has not been reported in other studies so far. In contrast, two mutations, V38A and N42T, were associated with a loss of CD4 cells during follow-up after enfuvirtide failure. Our study and the one of Svicher *et al.*[10] are difficult to compare. Indeed, even if both studies involved heavily treated patients, there were noteworthy differences in baseline characteristics such as the CD4 cell count, the CD4 nadir and plasma HIV-1 viral load. Furthermore, *gp41* was not sequenced at the same time points in the two studies. Further studies are, therefore, required to clarify the possible impact of *gp41* mutations on the CD4 cell count. The putative effect of *gp41* mutations on CD4 count evolution was described mainly after lengthy enfuvirtide selective pressure and might not have to be taken into account as a therapeutic strategy according to the new guidelines for antiretroviral use.

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Transparency declarations

None to declare.

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