

# KINETICS OF DISAPPEARANCE OF ENFUVIRTIDE-RESISTANCE HIV-1 MUTATIONS AFTER DRUG DISCONTINUATION

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## BACKGROUND

- Several studies showed a **rapid reversion** of HIV-1 genotypic resistance profiles to Enfuvirtide (ENF) to wild-type viruses;
- Genotypic reversion was observed **within the three months following drug interruption**;
- Although "bulk" sequencing revealed an apparent reversion, ENF-resistant viruses can persist in low proportions not detectable by "bulk" sequencing, and still be present as minority viral population;
- In seven patients, who stopped ENF after virological failure, we have **evaluated quantitatively, by molecular cloning analysis, the kinetics of disappearance of ENF-resistant viruses after ENF-discontinuation.**

## PATIENTS AND METHODS

- Seven patients displaying an immuno-virological failure under ENF therapy;
- Median ENF treatment duration: 6.4 months (range: 3-14 months);**
- HR1-HR2 gp41 domains "bulk" sequencing** determined from plasma samples at baseline, during ENF-therapy, and at all time-points available following ENF-discontinuation;
- Reported resistance mutations according to the ANRS AC11 2006 genotypic interpretation resistance rules as listed in [www.hivfrenchresistance.org](http://www.hivfrenchresistance.org);
- Molecular cloning (pCR4-TOPO) was performed at two time-points following ENF-discontinuation:**
  - last time-point with ENF-resistance mutations detectable by "bulk" sequencing;
  - first time-point with ENF-resistance mutations no more detectable by "bulk" sequencing;
- At least 20 to 30 clones were analyzed at each time-point.

## RESULTS

- Genotypic profiles of ENF-resistance at ENF-discontinuation were as follow:

**V38A in 3 patients;**  
**V38A-N42T-N43D in 1 patient;**  
**N43D in 2 patients;**  
**N43K in 1 patient.**

- "Bulk" sequencing of HR1 domain was performed from longitudinal clinical samples

Time after ENF-discontinuation (months)	Patients						
	A	B	C	D	E	F	G
Baseline	42S	WT	WT	WT	42S <sup>b</sup>	WT <sup>a</sup>	Not Available
Under ENF	<b>38V/A-42S</b>	<b>43D</b>	<b>38V/A-42N/T-43N/D</b>	<b>43K</b>	<b>38V/A-42N/S</b>	<b>38A<sup>c</sup>-42S</b>	<b>43D</b>
M1	<b>38V/A-42S</b>	<b>43D</b>	<b>42N/T</b>	<b>43K</b>	<b>38V/A-42N/S</b>	<b>38V/A/M/T-42S</b>	<b>43D</b>
M2		WT			<b>38V/A-42N/S</b>	<b>38V/A/M/T-42N/S</b>	<b>43D-44L/M</b>
M3	<b>42S</b>	WT		<b>43K</b>	<b>42N/S</b>		
M5	<b>42S</b>				<b>42N/S</b>	<b>42N/S</b>	
M6				WT	<b>42N/S</b>		<b>43N/D-44L/M</b>
M8					<b>42N/S</b>		
M9			WT	WT			
M11				WT			
M12			WT	WT	42S	WT	
M13				WT			
M14					42S		
M16				WT			
M17				WT	42S		
M20					<b>42N/S</b>		
M21				WT			
M22				WT			
M25				WT			

<sup>a</sup> WT: Wild-type.

<sup>b</sup> N42S polymorphism not implicated in ENF-resistance is shown in italicface.

<sup>c</sup> Resistance mutations are shown in boldface.

Blue-framed time points correspond to time points at which clonal analysis where performed.

**Table 1:** ENF-resistance profiles from plasma by "bulk" sequencing at baseline, under ENF, and at various times after ENF-discontinuation

- No association was observed between a given ENF-resistance mutation and the kinetics of mutation disappearance.

**The persistence of ENF-resistant minority viral populations was positively correlated to ENF treatment duration (r=0.84, p=0.02).**

- Clonal analysis showed:

(i) **complete genotypic reversion for 3/7 patients**, observed at 1, 3, and 9 months after ENF-discontinuation for patients B, A, and C, respectively;

(ii) **persistence of ENF-resistant viruses as minority viral population for 2/7 patients**; in a proportion of 5% and 8% detected 6 and 3 months after ENF-discontinuation for patients D, and E, respectively;

(iii) **persistence of ENF-resistant viruses as majority viral population** in a proportion of 24% and 88% for 2/7 patients, detected 5 and 6 months after ENF-discontinuation for patients F, and G, respectively;

ENF-treatment duration (months)	Patients						
	A	B	C	D	E	F	G
ENF-resistance mutation	V38A	N43D	V38A-N42T-N43D	N43K	V38A	V38A	N43D
Months after ENF-discontinuation							
1	44%	100%	20%				
2		0%					
3	0%			81%	8%	97%	
4							
5						24%	
6				5%			88%
7							
8							
9			0%				

Undetectable

Minority viral population

Majority viral population

**Table 2:** Proportion of ENF-resistant viral populations, obtained by clonal analysis, after ENF-discontinuation.

## CONCLUSIONS

- Clonal analysis approach of the kinetics of disappearance of ENF-resistance mutations after drug discontinuation showed that a **high proportion of resistant clones persist in only patients who had received ENF for long periods, more than six months (8 and 14 mths for study patients) and who displayed a persistent viral replication under ENF therapy**;
- Further investigations are needed to determine whether HIV-1 resistance to ENF generated during long-term treatment could facilitate persistence of resistant variants.