Impact of long term supervised Treatment Interruption (TI) in Patients with High CD4+ Cell Count on PBMC DNA Genotype

I. Pellegrin*[1], R. Thiébaut[2], P. Blanco[3], P. Merel[1], JF Viallard[3], MH Schrive[1], HJA Fleury[1], JF Moreau[4], and JL Pellegrin[3]

Department of Virology, Bordeaux University Hospital, France[1]; INSERM EO338, Bordeaux University Hospital, France[2]; Internal Medicine Infectious Diseases, Bordeaux University Hospital, France[3]; and Immunology, Bordeaux University Hospital, France[4]

Modified ABSTRACT

Background

Many patients (pts) with chronic HIV infection with long-term controlled viremia and high CD4+ count asked to stop HAART because of side effects. Many pts on HAART would not have been started on therapy based on today's more conservative guidelines. We present 6 months follow-up of PBMC DNA and plasma RNA loads and genotypes on these pts in whom treatment interruption (TI) was decided.

Patients and Methods

57 pts were consecutively included in this prospective pilot-study. At the time of CD4+ driven-TI (M0): 28 pts had HIV RNA<50 copies/ml for 40 [30; 56] months and 841 [644-1063]/µl CD4+ (group I); 29 had 3.3 [2.6; 4] HIV RNA copies/ml, 630 [537-748]/µl CD4+ with a median pre-HAART CD4+ cell count of 416 [336; 532]/µl (group II). Proviral DNA extracted from patients PBMC at M0 and M6 after TI was quantified by real-time PCR and analysed according to the IAS list 2002 for reverse transcriptase (RT) and protease (PR) genotypes.

Results

Considering the entire population at M0, median CD4+ count, plasma HIV RNA and proviral DNA loads were 739 [584-962] cells/µ, 1.9 [1.7-3.3] log_{10} copies/ml and 2.6 [2-3] log_{10} copies/10⁶ PBMC, respectively. A 6 months-TI was associated with a median decrease of 233 [127-346] CD4+/µl, an increase of 1.9 [1.2-2.8] log_{10} HIV RNA copies/ml and an increase in DNA load of 0.65 [0.13-1.14] log_{10} copies/10⁶ PBMC. Changes in HIV RNA and CD4+ count were significantly higher in group I than in group II (*P*< 0.04 and <10⁻⁴).

When considering M0 mutated PBMC HIV sequences, changes at M6 were as follows: *for RT sequences*: 32.3% lost 1-4 mutations without achieving WT genotype, 23.5% remained unchanged with 2 to 6 mutations, finally 44% shifted to wild-type; *for PR sequences*: no sequence shifted to WT, 28.6% lost 1-2 major mutations, 8.6% lost 1-3 polymorphic mutation, 62.8% remained unchanged with 1-3 mutations at polymorphism position.

Plasma genotyping in group II pts showed: *for RT sequences*: a shift from mutated to WT in 66.6% sequences, a lost of 1 to 6 mutations in 23.8% and no change in 9.5%; *for PR sequences*: no sequence shifted to WT, 47% lost 1-3 major mutations, 53% remained unchanged with 1 to 3 mutations at polymorphism position.

At M6, 3 pts had resumed treatment.

Conclusions

Within 24W of TI, we observed: 1) viral replication replenishes the pool of latently infected cells 2) Despite the repopulation of sensitive virus in plasma and the loss of some mutations in PBMC within 6 months of STI, resistant HIV-1 still persist in PBMC.

INTRODUCTION

The limitations of the drugs used against HIV include their toxicity, their tolerability, their propensy to induce resistance when not taken with absolute consistency, and their cost.

In patients with high CD4+ cell counts (patients receiving treatment of chronic infection with controlled viremia and patients who are receiving HAART now in whom treatment would not have been started based on current guidelines), we evaluated the kinetics of HIV RNA and DNA loads and of resistance mutations in PBMC and plasma after a 6 months period of TI.

PATIENTS AND METHODS

❑ ***Study population***

57 pts were consecutively included in this prospective pilot-study.

At M0 TI, 28 pts had HIV RNA<50 c./ml since 38 [30; 48] months and 841 [644-1063]/µl CD4+ (group I); 29 had 3.3 [2.6; 4] HIV RNA copies/ml, 630 [537-748]/µl CD4+ (group II).

History of previous ART regimens, kinetics of CD4⁺ cell counts and HIV-1–RNA plasma load since the first ART regimen and during the entire follow-up were collected. Results are presented Table 1

Blood samples were drawn into EDTA for CD4⁺ count, HIV RNA quantitation, preparation of 10⁶ PBMC aliquots before use for HIV DNA quantification and analyses of its RT and PR genotypes (M0, M3, M6).

❑ ***HIV-1–RNA quantitation***

Plasma HIV-1–RNA levels were determined using bDNA assay (Versant™ HIV-1 RNA 3.0, Bayer, Eragny, France).

❑ ***Quantification of HIV DNA with a Real-Time quantitative PCR (RT-PCR)***

Proviral DNA was extracted from patients PBMC at M0 and M6 after TI DNA using the High Pure Viral Nucleic Acid Kit (Roche Diagnostics, Gmbh, Mannheim, Germany) and was quantified by RT-PCR (LTR gene on LightCycler, Roche Biochemichal). The HIV-1 provirus load was expressed as the number of HIV-1–DNA copies/10⁶ PBMC (threshold, 1.8 log_{10} copies/10⁶ PBMC).

❑ ***RT and protease sequence analyses.***

Proviral DNA and plasma RNA RT gene (nucleotides 1–230) and the entire PR gene were sequenced using the CEQ2000 DNA sequencer (Beckman Coulter Inc., Fullerton, CA). We used the IAS list 2002 to consider mutations associated with antiretroviral resistance.

RESULTS

Table 1. Patient characteristics at inclusion.

Characteristics	All patients (n = 57)	CV<50 (n = 28)	CV>50 (n = 29)	*P*
Age (years)	44 [39; 51]	44 [41; 51]	43 [39; 51]	0.88
Sex (M/F)	41/16	9/19	7/22	0.50
Previous treatment				
Time from 1st ARV regimen (years)	6 [4.2; 7.5]	5.53 [3.6; 8.1]	6.32 [5.1; 7.3]	
Time from 1st HAART (years)	4.2 [3.4; 5.6]	3.64 [3.1; 5.2]	5 [3.8; 5.8]	
Nb of previous lines	3 [2; 4]	2 [2; 4]	3 [2; 3.5]	0.74
Previous mono-bitherapy, n (%)	25/54 (46%)	11 (44%)	7 (24%)	0.12
At the time of 1st ARV treatment				
CD4+ cell count (cells/µl)	416 [336; 577]	408 [332; 633]	416 [336; 532]	0.37
HIV RNA (log10 copies/ml)	4 [3.6; 4.7]	4.1 [3.1; 4.8]	4 [36.7; 4.4]	0.91
Duration of viral suppression (months)	–	40 [30, 54]	–	
Nadir CD4+ (before TI) (cells/µl)	345 [234; 483]	335 [227; 508]	357 [248; 430]	0.64
Zenith of plasma HIV RNA (log10 copies/ml)	4.24 [3.6; 4.7]	3.85 [3.2; 4.78]	4.26 [3.7; 4.6]	
ARV regimen at TI, n (%)				
2 NRTI + 1 IP	5 (10)	1 (3.8)	4 (16.7)	
2 NRTI + 1 NNRTI	19 (38)	14 (53.8)	5 (20.8)	
3 NRTI	9 (18)	6 (23)	3 (12.5)	
2 NRTI	10 (20)	2 (7.7)	8 (33.3)	
other	7 (14)	3 (11.5)	4 (16.7)	

P value was determined using the U-Mann et Whitney test, *P* value <0.05 was considered statistically significant.

2 patients resumed HAART at M2 because they were under stress and 1 had to restart treatment at M1 after a primary-infection symptomatology in association with thrombocytopenic purpura.

Table 2:
Kinetics of immunological and virological parameters after 6-months TI.

	All patients (n = 57)	CV<50 (n = 28)	CV>50 (n = 29)	*P*
▲ M6–M0				
▲ CD4⁺ (cells/µl)	−233 [−346; −127]	−299 [−427; −187]	−164 [−276; −84]	0.04
▲ HIV RNA (log_{10} copies/ml)	1.9 [1.24; 2.78]	2.6 [2.1; 2.93]	1.25 [0.67; 1.36]	$<10^{-4}$
▲ HIV DNA (log_{10} copies/10^6 PBMC)	0.65 [0.13; 1.14]	0.61 [0.11; 1.14]	0.69 [0.19; 1.16]	0.74

Disappearance of resistance mutations *in PBMC HIV proviral DNA* during a 6 months STI period. (Table 3)

When considering mutated sequences at M0, changes at M6 were as follows:

❑ *for RT sequences*:
- ✓44% shifted to wild-type
- ✓32.3% lost 1–3 mutations without achieving WT genotype
- ✓23.5% remained unchanged with 2 to 6 mutations.

❑ *for PR sequences*:
- ✓no sequence shifted to WT
- ✓28.6% lost 1–2 major mutations
- ✓8.6% lost 1 polymorphic mutation
- ✓62.8% remained unchanged with 1 to 3 mutations at polymorphism position.

❑ Most sequences which shifted were isolated from group I patients

Table 3

PBMC DNA genotype	All patients		CV<50, n=30		CV>50, n=27		P
Resistance related mutations	J0	M6	J0	M6	J0	M6	
RT	n=56	n=44	n=30	n=27	n=26	n=20	
WT	16	27	14	21	3	6	0.007 / 0.002
1-3 NRTI/ NNRTI mutations	27	17	14	5	13	12	
4-5 NRTI/ NNRTI mutations	10	1	1	0	9	1	
6-7 NRTI/ NNRTI mutations	3	1	0	0	3	1	
PR	n=55	n=42	n=28	n=24	n=26	n=24	0.35 / 0.32
WT	14	16	9	10	5	6	
1-3 mutations at polymorphism positions	28	32	16	14	12	16	
1-3 major mutations +/- polymorphism mutations	12	5	3	0	9	2	
▲ M6–M0	n=34		n=17		n=17		0.003 / NA
RT							
-No change	8 (23.5%)		5		3		
- Disappearance of 1-2 mutations without shift to WT	11 (32.3%)		1		10		
- Shift to WT	15 (44%)		11		4		
PR	n=35		n=18		n=17		
- No change	22 (62.8%)		13		9		
- Disappearance of 1-3 polymorphism mutations without shift to WT	3 (8.6%)		2		1		
- Disappearance of 1-2 major mutations without shift to WT	10 (28.6%)		3		7		
- Shift to WT	0		0		0		

P pour distribution des souches sauvages selon groupe 1 ou 2 à M0/M6. Amino Acid variants at polymorphic positions, i.e L10F/I/R/V, K20M/R, M36I, L63P, A71I/L/V/T, V77I. Major PI-related mutations among the following: V82I (n=2), G73S (n= 2), L90M (n=2), M46I (n=1), I54V (n=1), N88D (n=1).

Disappearance of resistance mutations and reappearance of wild-type genotypes ***in PLASMA HIV RNA*** during a 6 months STI period (in group II patients) (Table 4)

Table 4

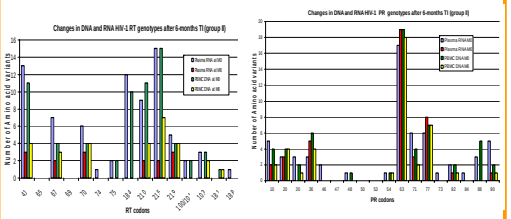
HIV resistance related mutations	PLASMA M0	PLASMA M6	PBMC M0	PBMC M6
RT, n (%)	*n=25*	*n=22*	*n=26*	*n=20*
WT	1 (4)	10 (66.6)	3 (11.5)	6 (30)
1-3 NRTI/ NNRTI mutations	11 (44)	4 (28.6)	13 (50)	12 (60)
4-5 NRTI/ NNRTI mutations	10 (40)	1 (7.1)	9 (34.6)	1 (5)
6-7 NRTI/ NNRTI mutations	3 (12)	0 (0)	3 (11.5)	1 (5)
PR, n (%)	*n=26*	*n=25*	*n=26*	*n=24*
WT	8 (30.8)	5 (20)	5 (19.2)	6 (25)
1-3 mutations at polymorphism positions	8 (30.8)	19 (76)	12 (46.1)	16 (66.6)
1-3 major mutations +/- polymorphism mutations	10 (38.4)	1 (4)	9 (34.6)	2 (8.3)
▲ M6–M0				
RT, n (%)	*n=21*		*n=17*	
No change	2 (9.5)		3 (17.6)	
Disappearance of 1-2 mutations without shift to WT	5 (23.8)		10 (58.8)	
Shift to WT	14 (66.6)		4 (23)	
PR, n (%)	*n=17*		*n=17*	
No change (persistence of 1-3 polymorphism mutations)	9 (53)		9 (53)	
Disappearance of 1-3 polymorphism mutations, no shift to WT	0		1 (5.9)	
Disappearance of 1-2 major mutations, no shift to WT	8 (47)		7 (41.1)	
Shift to WT	0		0	

❑ *for RT sequences:*
- ✓ 66.6% sequences shifted from mutated to WT
- ✓ 23.8% lost of 1 to 6 mutations
- ✓ no change in 9.5%

❑ *for PR sequences:*
- ✓ no sequence shifted to WT
- ✓ 47% lost 2-4 major mutations
- ✓ 53% remained unchanged with 1 to 3 mutations at polymorphism position.

❑ *Detection of RT + PR mutations*
- ✓ at M0, nb of mutations in plasma ≥ mutations in PBMC
- ✓ at M6, nb of mutations in plasma ≤ mutations in PBMC: drug-resistant virus was detected in PBMC after the plasma virus had shifted to drug-sensitive virus

Disappearance of resistance mutations according to codon positions (in group II patients) (Figure 1a 1b)



✓ Disappearance of mutations in RT between M0 and M6 concerned all codons and was more dramatic in plasma than in PBMC.

✓ Significant differences were found between major-PI and secondary-PI. In the PR, most of the major mutations converted to wild-type within 6 months. In contrast, only few of the secondary mutations disappeared during the same time period.

DISCUSSION-CONCLUSION

A 6 months-TI in patients with high CD4+ count was associated with:

❑ Rebound of HIV RNA and decrease in CD4+ cell counts higher in patients with undetectable HIV RNA at M0

❑ Viral rebound replenished the pool of latently infected cells

❑ Kinetics of drug-related mutations after cessation of HAART:
- ❑ Considering PBMC DNA sequences in the whole population:
  - ✓ shift to WT occured in 44% RT
  - ✓ disappearance of major PI-related mutations in 28.6% PR
- ❑ In group II patients
  - ✓ Plasma RNA RT: shift to WT occured in 66% of sequences
  - ✓ PBMC DNA RT: shift to WT occured in only 23% of sequences
  - ✓ In the protease: significant differences between major-PI and secondary-PI: most of the major mutations converted to wild-type within 6 months. In contrast, only few of the secondary mutations disappeared during the same time period.

This kinetics illustrated impact of these AmAc changes on viral fitness.

❑ Despite the repopulation of sensitive virus in plasma and the loss of some mutations in PBMC within 6 months of TI, resistant variants still persist in PBMC and could reemerge, once therapy is reintroduced, by activation of cell reservoirs that bear resistance mutations, when the virus replication is not sufficiently suppressed by the new drugs.

❑ Both M0 plasma HIV RNA (detectable viral load) and PBMC HIV DNA (undetectable viral load) genotypes should be taken into account to guide decisions about subsequent therapy after TI.