

## Viral and immunologic follow up of 4 to 9 years of AIDS treatments by quadruple combinations of virostatics including integrase inhibitors applied in short sequences differing by drug rotation

Georges MATHÉ<sup>1</sup>, Christian MORETTE, Michel HALLARD, Paulo PONTIGGIA, Denise BLANQUET, Françoise HAGE

(Institut de Cancérologie et d'Immunologie & Hôpital Suisse de Paris BP 60-92133 Issy les Moulinaux, France)

**KEY WORDS** retroviridae; integrase inhibitors; ellipticines; acriflavine; vitamin B12; HIV-1

### ABSTRACT

**AIM:** To present the 4 to 9 years (median: 6 years) treatment follow up of 10 HIV<sub>1</sub>-AIDS patients, 9 at AIDS and 1 at A<sub>3</sub> stages. **METHODS:** We have applied from 1992 to 1994, AZT combined with 2 integrase inhibitors, acriflavine and hydroxy-methyl-ellipticine. We could shift, in 1994, to combinations of 3 drugs including two more retrotranscriptase inhibitors (RTI), ddI and ddC, and, after 1995, to combinations of 4 drugs including also two other RTI, d4T and 3TC, and 3 protease inhibitors (PI), indinavir, ritonavir, and saquinavir. In 1998, as cobalamine was shown by an *in vitro* test, to act as integrase inhibitor, vitamin B12 was added in cycles of various lengths. Every three weeks, not only the investigations were repeated, but the virostatics were changed. **RESULTS:** No grade 2 virostatics toxicity has been registered. The viral loads (VL) decreased according to exponential curves. Their initial parts obeyed first order kinetics. The second parts were and still are asymptotic. The first parts could be rectilinear or sinuous. The sinuosities were associated to cofactors present before treatment (chimerism, UV irradiation, hepatitis C or B and C, brain toxoplasmosis). The asymptotic parts, whose VL were below PCR detectable

levels, presented discrete, reversible HIV<sub>1</sub> rebounds, associated to other cofactors (such as herpes zoster, herpes 6, CMV, flat condyloma, and influenza). Among immunologic parameters, the monocyte and CTL numbers increased and presented, during the rapidly decreasing part of VL curve, a significant inverse correlation with it. Neither CD4<sup>+</sup> nor suppressor T-cell (STC) numbers presented such correlation. Near 100 % of CTL were CD28<sup>+</sup>. Later, vitamin B12 applications increased monocyte and CD28<sup>+</sup> CTL numbers, and appeared to reinforce VL stabilization. **CONCLUSION:** The combinations of inhibitors affecting 3 retrovirus targets, retrotranscriptase, integrase, and protease have given to 10 out of 10 AIDS patients survivals varying today between 4 to 9 years, in excellent conditions. The UVA-pretreated patient is the only one presenting a not maximally reduced asymptotic VL, while his CD4<sup>+</sup> and STC have been absent for 8 years. Other patient VL regressed exponentially to become asymptotic, below PCR detectable levels.

### INTRODUCTION

Between 1988 and 1992, many HIV<sub>1</sub>-infected patients had reached the AIDS phase and had become resistant to zidovudine (AZT) discovered in 1986<sup>[1,2]</sup>. They were however receiving it from the experts, as it was the only available HIV<sub>1</sub> officially recognized virostatics.

There was an imperative need for new virostatics. As AZT is an inhibitor of retrotranscriptase (RT)<sup>[1]</sup>, an early target in the virus cycle, we inferred that the most desirable second HIV<sub>1</sub>-virostatics should be an inhibitor of retrovirus integrase, the next target after RT along this virus cycle. Fujiwara had devoted to it, an excellent

<sup>1</sup> Correspondence to Prof Georges MATHÉ.

Phn 33-1-41-331-255. Fax 33-1-41-331-274.

**Abbreviations** ACF: acriflavine; AZT: zidovudine; ddC: zalcitabine; ddI: didanosine; HEL: hydroxy-methyl-ellipticine; IDV: indinavir; RTV: ritonavir; SQV: saquinavir; 3TC: lamivudine; d4T: stavudine

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review in 1988<sup>(3)</sup>. It is the enzyme which involves a coordinated joining of the two ends of a proviral DNA molecule, into precisely spaced sites of host DNA. It mediates a concerted strand cleavage ligation between the two half substrates, at one or both viral DNA joining sites<sup>(4)</sup>. Because the present paper publishes the first therapeutic, clinical use of integrase inhibitors, we must recall their discovery and preclinical study.

**Hydroxy-methyl-ellipticine and acriflavine shown in 1989, to be retrovirus integrase inhibitors** For the screening of retrovirus integrase inhibitors (RVII), we used murine Friend's virus because this retrovirus produces in mice of some genotypes (such as DBA/2), erythroblastosis, induced by proviral insertions into *c-erb B*<sup>(5,6)</sup> (while it produces immunosuppression in others, such as Rfv-3r/s<sup>(7)</sup>). Our test was based on the quantitative inhibition of erythroblastosis. We had used it in 1964, as a virostatic test to evaluate the power of bone marrow graft reaction against leukemia inducer viruses (GvH)<sup>(8,9)</sup>.

We had inquired about series of chemicals which would affect provirus DNA at the two ends of its genome<sup>(10)</sup>. These two ends are tightly associated with the terminal proteins, essentially converting the linear genome into a topologically constrained cycle 4. One of the series submitted was composed of ellipticine and some of its derivatives, methoxy-9-ellipticine (M9E) (which we had shown, in 1970, to be moderately onco-static<sup>(11-13)</sup>, and hydroxy-methyl-ellipticine (HEL) (to

which Paoletti had attributed the same action<sup>(14)</sup> and commercialization, Celliptium<sup>®</sup>). The second series comprised analogues of aminoacridines, among which acriflavine (ACF) had been applied as systemic bacteriostatics in patients suffering from gonococci<sup>(15,16)</sup>. It has also been shown to be acting on *Pneumocystis carinii*<sup>(17)</sup>, and as a multivalent strong local microbiostatics (which is commercialized in France, Chromargon<sup>®</sup>, Richard).

Both ellipticine derivatives, M9E and HEL, appeared to exert a strong anti-retrovirus action. We chose HEL<sup>(18)</sup> (Fig 1a), as its optimally efficient dose in mice was 10 000 more active than that of AZT taken as control. Among amino-acridine analogues, only acriflavine (ACF) (Fig 1b, 1c) and proflavine were active<sup>(19)</sup>. The maximally efficient dose of the first one, was 10 times more active than that of AZT<sup>(19)</sup>.

Though HEL had been applied to hundreds of cancer patients<sup>(14)</sup>, and ACF to thousands of gonococci infected subjects<sup>(15,16)</sup>, we repeated their experimental and clinical development in the mouse for the anti-viral use, and, in four species as advised by Freireich<sup>(20)</sup> (mouse, rat, rhesus, monkey, and dog), their comparative, toxicologic, investigation<sup>(21,22)</sup>. The optimal doses extrapolated from the above quoted ones, were perfectly tolerated, and both products appeared to be HIV<sub>1</sub> virostatic at doses which were below those respectively necessary to be active against cancer for HEL<sup>(14)</sup>, and gonococci for ACF<sup>(15,16)</sup>.

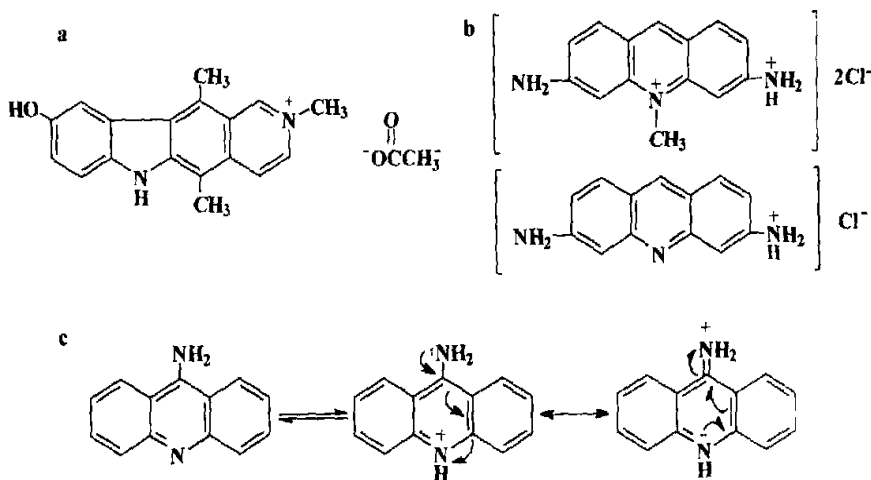


Fig 1. a) Ellipticum acetate [6H-pyrido[4,3-b]carbazolium, 9-hydroxy-2,5,11-trimethyl-acetate]. b) Acriflavine chloride (acriflavinium, 3,6-diamino-10-methyl-chloride, monohydrochloride mixed with 3,6-acridinediamine monohydrochloride). c) High ionisation at physiologic pH, is necessary to the antibacterial and antiviral actions of acriflavine.

Between 1989 and 1992, we were asked by several patients having reached AIDS phase, and whose HIV<sub>1</sub> were resistant to AZT<sup>(21)</sup>, to be submitted to one of these agents or to both, in the framework of compassionate and individual treatments<sup>(21,22)</sup>. Adopting Freireich<sup>(23)</sup> Gehan's<sup>(24)</sup> rules for cancer drug studies, we registered a 50 % decrease of p24 antigen in 20 % of the patients (with  $P < 0.05$ )<sup>(21,22)</sup>. A similar decrease of VL will be found when its PCR evaluation became possible<sup>(22)</sup>. Both agents appear active on AZT resistant HIV<sub>1</sub><sup>(22,25)</sup>. The reduction of both viral parameters appear to obey, as cancer cells<sup>(26)</sup>, first order kinetics.

This led us to predict that any objective of the virus eradication would need virostatic combinations.

**Retrovirus integrase inhibitors combined to other virostatics** We had achieved experimental combinations on Friends virus<sup>(27)</sup>. At doses at which neither AZT, nor HEL, nor ACF, applied individually, were able to suppress erythroblastic colonies induced by infected plasma diluted to the tenth, the combination of the three agents did so. A combination of the 3 drugs at superior doses, could even suppress colonies induced by non diluted, infected plasma<sup>(27)</sup>.

In the above described experiment, we had only attacked two targets of the virus, defined by the two enzymes respectively determining retrotranscription and integration.

When the inhibition of HIV<sub>1</sub> integrase by HEL could be demonstrated by *in vitro* tests<sup>(28,29)</sup>, some experts worried about the fact that this agent cleaved DNA and inhibits topoisomerase II, thus attacked the host DNA and/or its associated enzymes<sup>(29)</sup>. Other experts could identically worry about acridine derivatives<sup>(30,31)</sup>.

Today, we know that retrotranscriptase inhibitors (RTI) also affect other HIV<sub>1</sub> enzymes<sup>(32)</sup> and other host targets<sup>(33)</sup>, such as nuclear mitochondrial enzymes<sup>(34)</sup>. The most severe adverse effect of RTI is the inhibition, especially by AZT, of telomerase activity, which irreversibly shortens telomeres<sup>(35,36)</sup>. Protease inhibitors, also conceived as specific attackers of this HIV<sub>1</sub> enzyme<sup>(32,33)</sup>, induce a severe lipid-glucid dysmetabolism<sup>(37,38)</sup>.

The rare and discrete toxicities, registered during the past uses of HEL and of ACF, hemolytic anemia for the first<sup>(14)</sup>, albuminuria for the second<sup>(15,16)</sup>, have not been observed in our patients<sup>(21,22)</sup>. No manifestation, possibly bound to their interactions with other drugs at the level of cytochromes P450<sup>(39,40)</sup>, have been mentioned.

HEL and ACF allowed us to start in 1993, to treat AIDS patients with virostatics combinations affecting two viral targets, when two more nucleosidic RTI, ddI and ddC, became available<sup>(32)</sup>. HEL and ACF contributed to increase to 10, the number of virostatics available in 1995, when two more RTI (d4T and 3TC) and three protease inhibitors, INV, RTV, and SQV, also became available<sup>(32)</sup>, and to increase to three, the number of attackable targets (Tab 1).

**Tab 1. The series, years of first availabilities, and common doses of the virostatics applied and the three types of their combinations according to these availabilities.**

Series	Years of first availabilities	Common doses of virostatics applied				
<b>Retrotranscriptase inhibitors</b>						
AZT	1986	250 mg <i>po</i> , 2 times/d				
ddI	1992	250 mg <i>po</i> , 2 times/d				
ddC	1992	0.75 mg <i>po</i> , 3 times/d				
d4T	1995	40 mg <i>po</i> , 2 times/d				
3TC	1995	150 mg <i>po</i> , 2 times/d				
<b>Retrovirus integrase inhibitors</b>						
ACF	1989	50 mg <i>po</i> , 1 time/d				
HEL	1989	50 mg <i>po</i> , 1 time/week				
<b>Protease inhibitors</b>						
IDV	1995	800 mg <i>po</i> , 3 times/d				
RTV	1995	600 mg <i>po</i> , 2 times/d				
SQV	1995	600 mg <i>po</i> , 3 times/d				
<b>Two drugs and three drugs out of 5 available from 1992 to 1995</b>						
AZT	ddC	ddI	AZU	AZT	ddI	AZT
ACF	HEL	ACF	HEL	ddI	ddC	ddC
				ACF	HEL	ACF
<b>Four drugs out of 10 available after 1995</b>						
AZT	ddC	AZT	d4T			
d4T	3TC	d4T	3TC			
ACF	HEL	ACF	HEL			
SQV	RTV	INV	SQV			

Hence between 1992 and 1996, we had successively combined two, then three virostatics out of 5 available. After 1995, we successively combined three, then four virostatics out of 10 available (Tab 1)<sup>(22,41)</sup>.

Though continuous treatment was considered as justified by the possibly and generally rapid turnover of HIV<sub>1</sub><sup>(42)</sup>, we proposed to apply this continuity according to a special model. We base it on the notion that cell targets comprise multiple fixing sites<sup>(43)</sup>, which can get rid of most drugs themselves, and repair growth back after a certain time. This led us to apply permanent and long pharmacological actions, by allocating them in

alternative fashion, which could avoid the establishment of cross and/or cumulative resistances, and of toxicities.

Tab 1 gives examples of such alternative applications, according to the numbers of virostatics which were available in the different periods of the treatment.

**Vitamin B12, a third HIV<sub>1</sub> integrase inhibitor applied to patients** Classical treatments of patients at AIDS phase, comprised a *Pneumocystis carinii* preventive treatment<sup>[33]</sup>; eight of our patients had received trimethoprim-sulfamethoxazole (TSXM) and two had received pentamidine. In 1997, we were impressed by persisting macrocytosis in all patients, which led us to search and detect hypofolatemia and hyperhomocysteinemia. The levels of vitamin B12 were normal.

Cobalamine<sup>[44]</sup> and its divalent metal, cobalt<sup>[45]</sup>, had just been revealed by *in vitro* tests, to act as HIV<sub>1</sub>-integrase inhibitors. We thus combined daily folic acid (15 mg/d) (indicated to quantitatively correct the folate deficit), to vitamin B12, applied at the dose of 1000 µg/d, as folate and vitamin B12 metabolisms are intimately meshed in the metabolic and genomic methylations<sup>[46]</sup>.

This complementary part of our patient treatment has not been less individualized than the basic one. Folic acid has been applied permanently, while vitamin B12 was administered in cycles, whose lengths varied.

## MATERIALS AND METHODS

**Toxicity safeguards** The toxicologic investigations concerning the feared adverse effects of all applied drugs, were repeated systematically each three weeks.

We only registered grade 1, minor manifestations, such as nausea, whose duration was inferior or equal to the 21 d sequence, and which disappeared as soon as the next sequence started.

When the lipidodystrophic and diabetic syndrome associated with continuous protease inhibitor treatment was published<sup>[37,38]</sup>, we noticed its absence in our patients. We are tempted to attribute a preventive effect to PI rotation. The patients have been prescribed a strict diet, including fish oil, known to avoid insulin resistance<sup>[47]</sup>.

No significant reduction of CD34<sup>+</sup> stem cell numbers has been registered, except in 2 patients (Fig 2); One is patient S, who had been submitted to a 6 month reinforcement of the virostatic numbers (he

received 6 instead of 4). The other is patient D1b, whose suppressor T-cell numbers had increased at the occasion of a herpes 6 infection; we had reduced them by a 10 d application of cyclophosphamide at the minimal dose of 50 mg/d.

**HIV<sub>1</sub> evaluation and general microbiological control** Before 1992, the evaluations were limited to the study of antibody by ELISA and Western blot methods, and to that of p24 antigen plasma levels. Qualitative PCR-viral load (VL) evaluation became available in 1992, and quantitative PCR in 1996. The Roche-Monitor equipment was used in the Pasteur-Cerba Laboratory, which was in charge of evaluating VL every three weeks. We only evaluated the antiretroviral drug resistance in the laboratory in the case of the UV intoxicated patient, the only patient who did not maximally reduce the VL<sup>[48]</sup>. We did not characterize the allele CCR2, either in the patients, or in their partners, which was now known to constitute a prognostic factor<sup>[49]</sup>.

The conventional investigations of microbiological cofactors were systematically performed according to all guides<sup>[33]</sup>.

**Immunological parameters** The immunologic evaluations were also repeated every three weeks. The parameters which have appeared to be the most sensitive, have been the numbers of the cytotoxic T-lymphocytes (CTL), as will be confirmed later by Oldstone<sup>[50]</sup>, and of their CD28<sup>+</sup> proportion and absolute numbers. The second test in sensitivity has been, before and especially under vitamin B12-folic acid applications, the monocyte counts. The counts of the regulatory cells (CD4<sup>+</sup> helper T-cells, and CD8<sup>+</sup>, CD57<sup>+</sup>, CD28<sup>+</sup> suppressor T-cells)<sup>[51]</sup>, have also been repeated every three weeks, as was the evaluation of global Ig, of IgM, IgA, and IgG levels. We only occasionally evaluated IL-1, IL-2, and IL-12 levels.

**The patients** As seen on Tab 2, we treated six homosexual males, two drug addicts, a heterosexual male who has replaced opiates by alcohol, and a heterosexual female who has discontinued opiates but has to receive various neuroleptics prescribed by her psychiatrist. This consumption induced a global obesity of 15 kg, which was established before protease inhibitors were used, and was neither aggravated nor improved by them. There were also two heterosexuals (a female and a male) who had been contaminated by partners whom they were considered as seronegative. Two of the homosexual

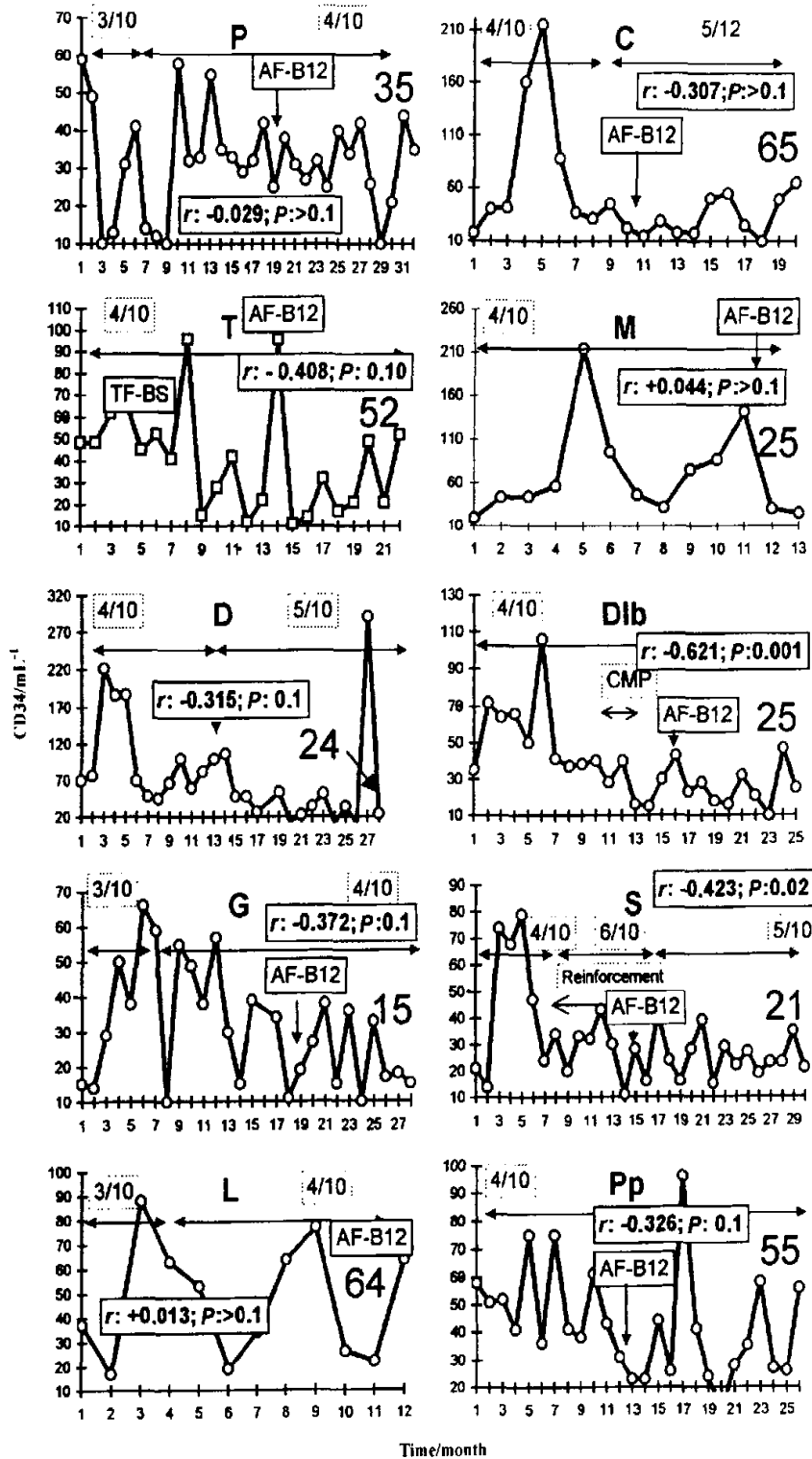


Fig 2. The level of blood CD34 were evaluated during several months. One notes their significant decrease in patient Dib who received, to reduce his suppressor T-cell number increase, 10 d of cyclophosphamide at 50 mg/d, and in patient S who received a reinforcement comprising 6 (instead of 4) of the same 10 virostatics available.

Tab 2. Summary of evolution.

	Patients initials	Date of birth	Sex	Etiology form	Initial cofactors	Seropositivity date	1st Treatment date	Numbers of virostatics included in sequential combinations among their total numbers available							
								2 \ 5		3 \ 5		3 \ 10		4 \ 10	
								First	Last	First	Last	First	Last	First	Last
<b>First group</b>															
CD4 counts	P	1963	M	Homo	Psoriasis U V A	1993	1993 Oct	105	82	56	19	17	45	21	22
	T	1957	M	Homo	B & C hepatitis	1992	1992 Nov	47	21	40	40	33	108	65	143
Viral loads (log 10)	P									5.3	5.9	5.3	5.2	5.7	5.2
	T									4.6	4.1	5.4	4.4	3	3.8
<b>Second group</b>															
CD4 counts	D	1957	F	Drug user	C hepatitis	1989	1995 Apr			140	102	192	192	348	628
	G	1956	M	Hetero	Chimerism	1993	1994 Mar			126	255	270	269	261	217
	L	1968	M	Homo		1991	1995 Jun			54	266	266	315	286	400
Viral loads (log 10)	D									6	4.5	5.8	5.8	4.1	<1.7
	G											4.3	3.3	3	<2.3
	L									5.8	4.7	4.5	<2.3	<2.3	<2.3
<b>Third group</b>															
CD4 counts	C	1963	M	Drug user	C hepatitis	1995	1995 Apr							129	331
	M	1966	M	Homo	Chimerism	1989	1995 Sep							201	400
	S	1961	M	Homo		1984	1997 Jan							393	775
	Dlb	1963	M	Homo	Toxoplasmosis	1994	1996 Dec							145	592
	Pp	1967	F		Asthma	1990	1997 Jun							10	200
Viral loads (log 10)	C													4.8	2.51
	M													5.3	<2.3
	S													4.6	<1.7
	Dlb													6	3.11
	Pp													5.9	1.95

patients (L, M) had to change their medical team for geographical reason; though they are in good condition, we shall limit their description and data to the duration of our own treatments.

All were at AIDS phase except one which was at the A<sub>3</sub> stage.

**Initial and/or early cofactors** The results of the initial treatments must be considered in relation to cofactors detected before treatment. The most severe cofactor was presented by patient P. It was the consequence of a PUVA dose of 214.5 J/cm<sup>2</sup> (spectrum 230 to 320 nm), prescribed by his dermatologist for a psoriasis, just before our treatment was applied.

Among the other early cofactors, two patients (D and C) were presenting hepatitis C, and a third one (patient T) was presenting both hepatitis C and B.

Patient G, a heterosexual male, presented, during one year, a blood chimerism characterized by the presence of typical female drumsticks in 51 % of his polymorphs, 3 % XX mitosis, and Gm incompatibilities.

Patient M, a homosexual male, presented Gm incompatibilities. None of the chimeric patients had been transfused.

**Case reports** We shall briefly describe four cases illustrating the most typical problems. The case of patient P (Fig 3) is dominated by one of his two initial cofactors, a psoriasis and the quoted PUVA administration which he received before and during the beginning of our treatment. The consequence has been a reduction to near 0 of his CD4<sup>+</sup> and his CD8<sup>+</sup>, CD57<sup>+</sup> (suppressor T) cell numbers, which persists today, after more than 7 years. His VL has regressed according to an exponential curve. Though its regression coefficient *r*, is -0.688, though the curve has reached an asymptotic phase, the VL of the latter is still at a rather high PCR evaluable level. His vitamin B12 cycle of 5 months increased the monocyte numbers, as in other patients. Its possible effect on VL does not appear on the data. He presented a late cofactor, a flat condyloma of the foreskin mucosa which completely disappeared after quercetin application.

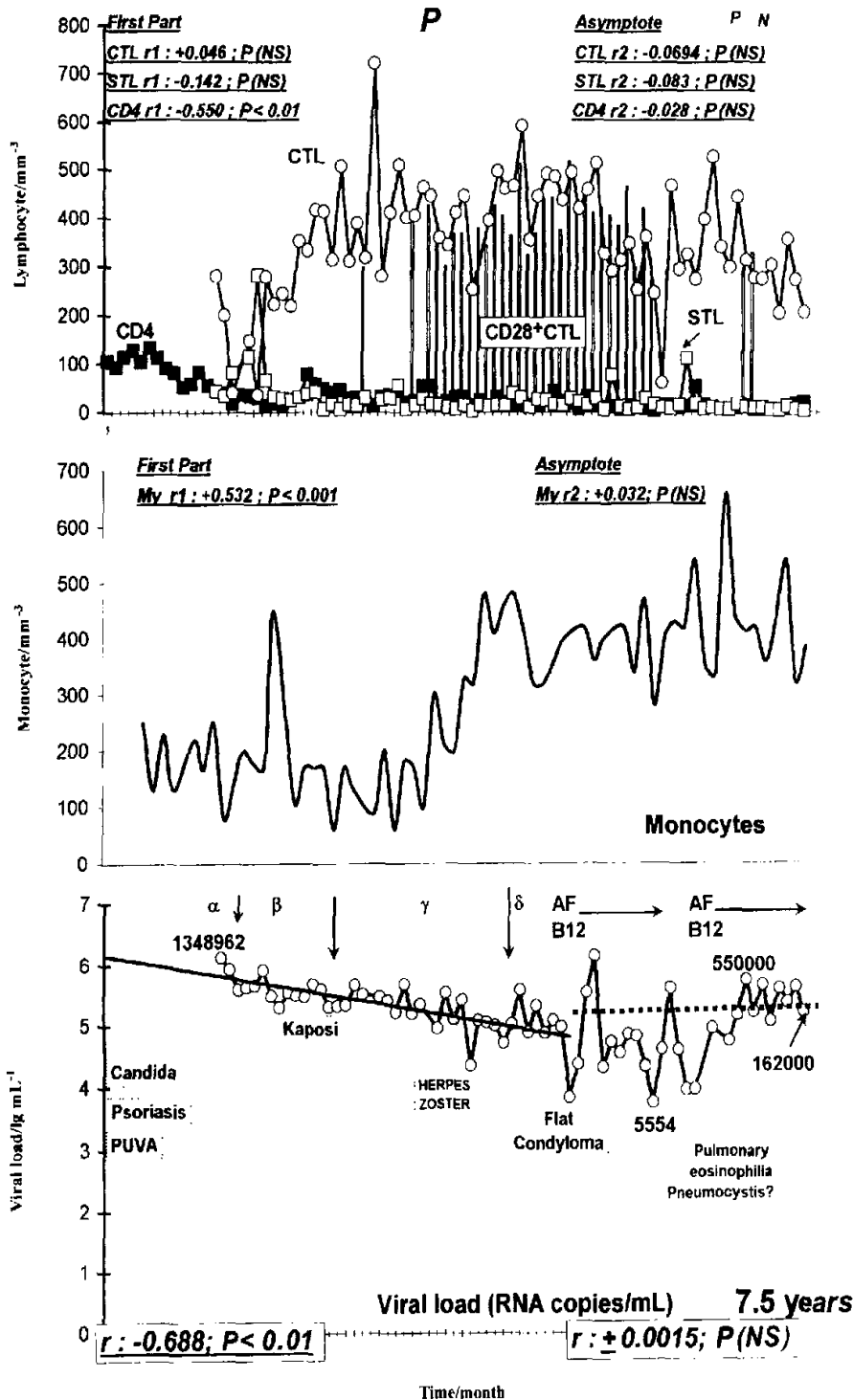


Fig 3. Individual curves of the viral loads and immunologic parameters of patient P. Victim of PUVA irradiation before he started our treatment, he had reduced his CD4<sup>+</sup> and his suppressor T-cells to near zero. This condition persists after 7.5 years.  $\alpha$  Designates the phase of treatment combining 2 virostatics out of 5 available;  $\beta$ , the phase combining 3 out of 5;  $\gamma$ , the phase combining 3 out of 10;  $\delta$ , the phase combining 4 out of 10. AF: folic acid continuous treatment; B12: one month first cycle of vitamin B12; CTL: cytotoxic T-lymphocytes; STL: suppressor T-cells. See the general comment of the case in the text. NS: not significant at  $P < 0.05$ .

Patient D1b (Fig 4) was presenting, when he started his quadruple virostatic therapy, a brain toxoplasmosis. He was cured by pyrimethamine combined with dapsone, and his VL regression was rapid. After VL decreased to PCR near undetectable level, it presented a discrete rebound, which led us to discover a herpes 6 infection. Interestingly, the latter was associated to a suppressor T-cell number increase, itself associated to a symmetric decrease of CTL. We reduced the suppressor T-cell number by the application of 10 d of cyclophosphamide at the dose of 50 mg/d.

His vitamin B12 first course was only one month. Because of allergic reactions, we replaced it by cobalt, which also inhibited integrase<sup>[46]</sup>. The monocyte number increase associated with vitamin B12, was not prolonged under cobalt application. But the increases of those of CTL and CD4<sup>+</sup> cells have been prolonged.

Patient Pp (Fig 5) had at the beginning of her treatment, 4 CD4<sup>+</sup> cell/mm<sup>3</sup> and less than 10 CTL. Her VL decrease to imperceptible PCR level was the most rapid among all patients ( $r$ : -0.971). She took vitamin B12 first course for 3 months and a half. Her monocytes, her CTL, CD28<sup>+</sup> CTL and CD4<sup>+</sup> numbers increased. This did not avoid two small and reversible HIV<sub>1</sub> rebounds associated with *Paramyxovirus influenzae*.

Patient S (Fig 6) presents the most remarkable result, probably because he was at stage A<sub>3</sub> when he started the quadruple treatment. No early or late cofactor was detected. No significant HIV<sub>1</sub> rebound appeared. His immunologic condition improved rapidly: his CTL and his CD28<sup>+</sup> CTL numbers increased. His CD4<sup>+</sup> and suppressor cell numbers are normalized. Vitamin B12 has increased his monocyte numbers more slowly than in other patients (which is possibly due to the mentioned virostatics reinforcement).

## RESULTS

**HIV<sub>1</sub> load exponential regression** HIV<sub>1</sub> load curves have regressed, whatever their initial levels, to a non PCR detectable number of RNA copies/mL, except in patient P, who has not yet, after 8 years of treatment, reached this level.

The reduction coefficient,  $r$ , of the first (rapid) part of VL exponential reduction, varies between -0.331 and -0.688 for the patients who started their treatments with combinations of 2 then of 3 virostatics, and between -0.462 and -0.971 for those who started

their treatments with the 4 drug regimen.

Patient C (whose  $r$  was -0.462) was presenting hepatitis C when he started his treatment. We can thus wonder whether his relatively small  $r$  is either associated with the presence of this initially manifested cofactor, or is associated with the fact that his initial combinations comprised small numbers of drugs.

### Immunologic parameters evolution

During the initial phases of the exponential VL curves, before they became asymptotic, the levels of the HIV<sub>1</sub> viral loads were significantly correlated with the CTL, the CTL CD28<sup>+</sup> and the monocytes blood counts (not shown). They were correlated neither with CD4<sup>+</sup> numbers, nor with those of suppressor T-cells, but they were with the positive or negative differences between both numbers (not shown).

We have evaluated several occasions, the levels of interleukins 1, 2, and 12. All have been normal in all evaluations, except those of IL-12, which were elevated in two patients presenting hepatitis C.

After vitamin B12 was added for a first cycle, whose lengths was variable between patients (from 1 to 9 months), we observed a rapid increase, abrupt in most subjects, of the monocyte numbers. We also observed in most cases, a more or less rapid increase of CTL, of CD28<sup>+</sup> CTL and of CD4<sup>+</sup> cell numbers. The viral load appeared to be stabilized below the minimally available level at PCR. It however did not avoid the appearance of the HIV<sub>1</sub>-rebounds associated with late cofactors.

**The asymptotic part of HIV<sub>1</sub> viral load curve, and the HIV<sub>1</sub> rebounds associated to late cofactors** As a matter of fact, the HIV<sub>1</sub> viral load levels, though they generally were below PCR detectable ones (either < 200 or < 20 RNA copies/mL), have been, except in patient S, associated with discrete, reversible HIV<sub>1</sub> rebounds.

We have carefully searched, every 3 weeks, between two sequences, and at the time of each rebound, all possible associated cofactors which could explain HIV<sub>1</sub> activation. We have found some in all cases of rebounds.

It is interesting to note that the appearance of these late persisting or acquired cofactors seems to be bound to their nature; Fungi have ceased to appear first, then the bacterias, while the DNA virus episodes may still appear (the three late cofactors presented by patient P, a herpes 8 associated and curable Kaposi sarcoma, a herpes zoster and a flat condyloma, illustrate this fact). The RNA



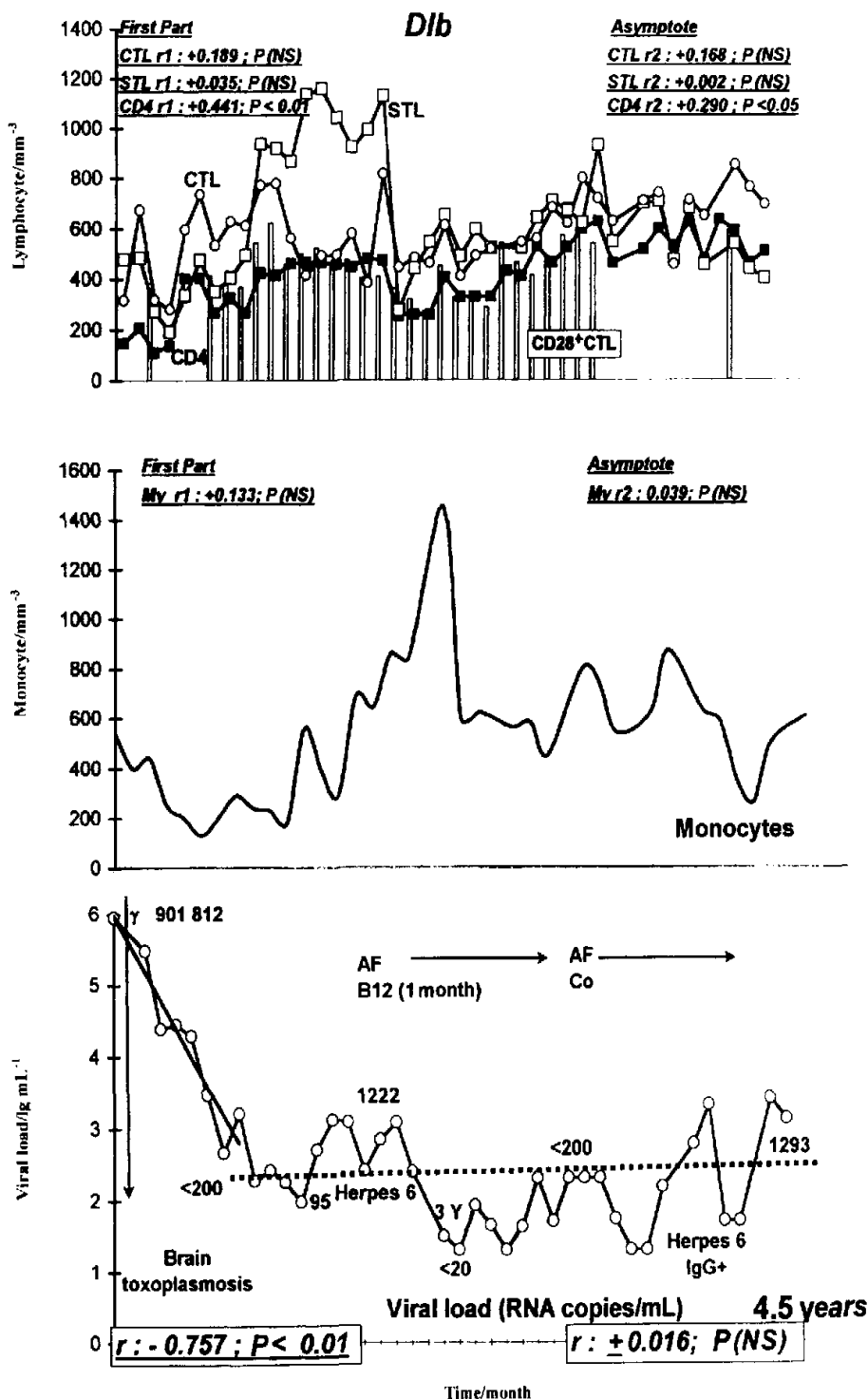


Fig 4. Individual curves of the viral loads and immunologic parameters of patient D1b. The abbreviations are the same as in Fig 3. As the next two other patients, patient D1b has been submitted, from the beginning of his treatment to the four drug regimen. He presented, one year and a half after the beginning of his treatment, a HIV<sub>1</sub> rebound, associated to suppressor cell increase, and to a symmetric CTL number decrease. The STC increase was interrupted by cyclophosphamide, applied for one week at small doses. These facts led us to a complete search of cofactors, which found herpes 6. NS: not significant at  $P < 0.05$ .

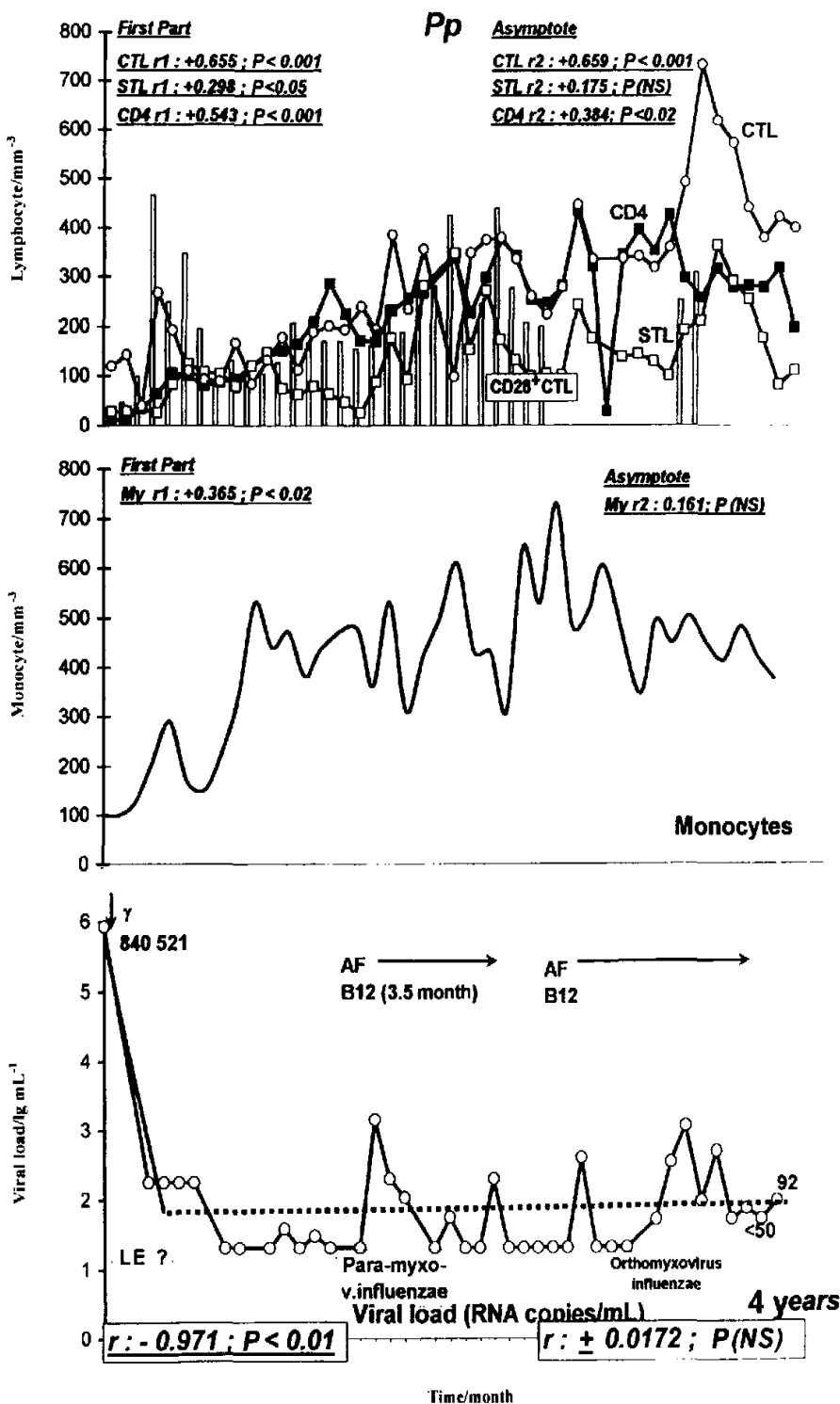


Fig 5. Individual curves of the viral loads and immunologic parameters of patient Pp. Note that the patient presented during her asymptote, two HIV<sub>1</sub> rebounds. The only and common cofactor found was a para-myxo-virus influenzae. As the previous patients, she presented, as soon as vitamin B12 was applied for a long cycle, an abrupt and significant increase of her monocyte numbers, which is associated with a tendency to a slope increase of the CTL and of the CD4. NS: not significant at  $P < 0.05$ .

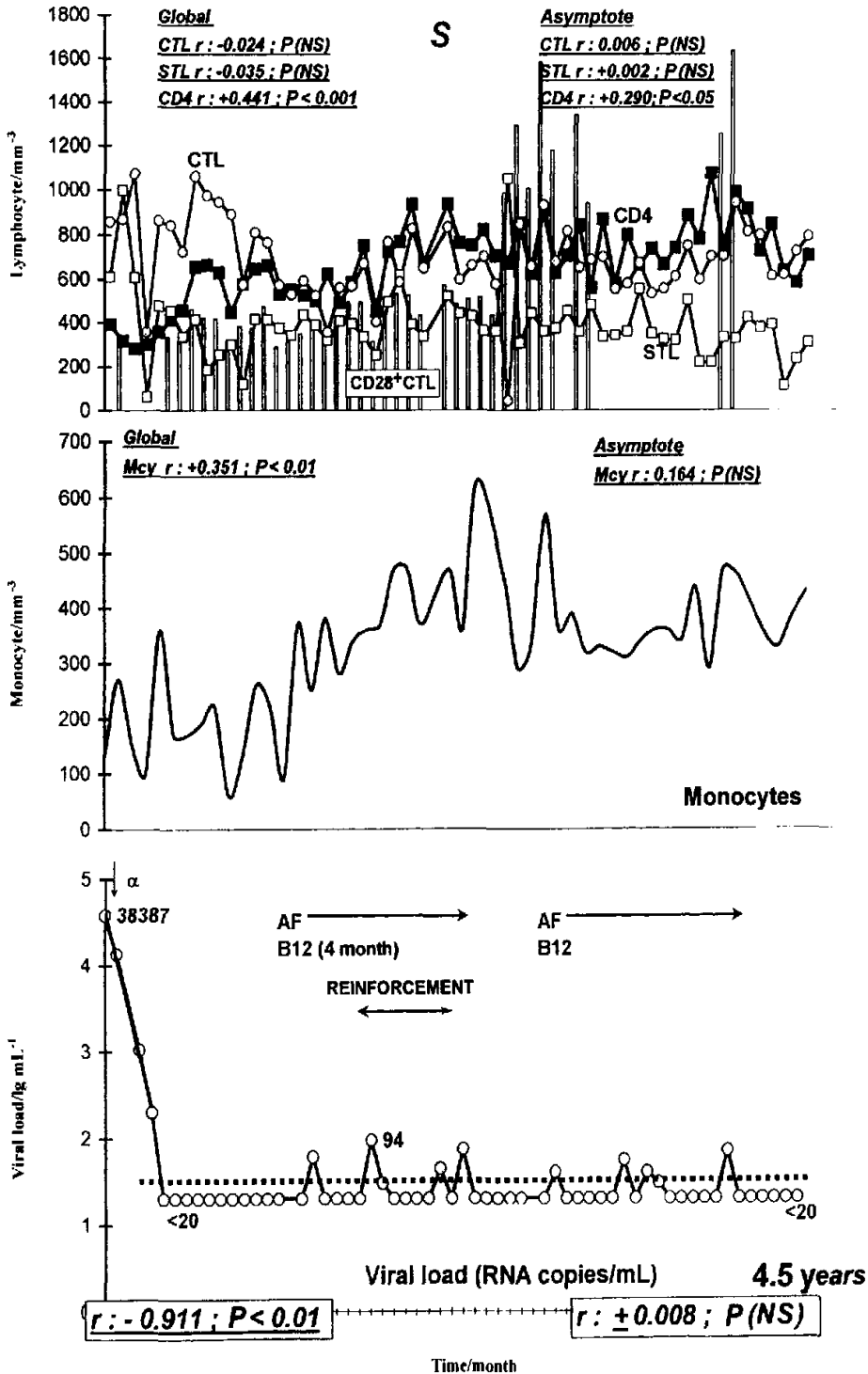


Fig 6. Individual curves of the viral loads and immunologic parameters of patient S. He was the only patient at the A<sub>3</sub> stage when he started his treatment. He has been the only patient who has not presented any significant HIV<sub>1</sub> rebound. In complement of the folic acid-vitamin B12 complex, he was the object of a virostatic reinforcement: He received, after the cycle of vitamin B12, a combination of six instead of four virostatics out of 10 available. His CD4<sup>+</sup> cell numbers are now normal, but he presented, after the reinforcement, a discrete but significant decrease of CD34<sup>+</sup> cell numbers. NS: not significant at  $P < 0.05$ .

viruses, such as that of chronic hepatitis C, have not presented any manifestation.

**Survival and quality of life** All patients are alive, which can be most favorably compared with the AIDS survival data provided by the NIP Panel on MACS<sup>[52]</sup> trial (Tab 3) and by a Vella study<sup>[53]</sup>. All our patients enjoy a normal and professionally active life.

**Tab 3. Survival of our patients compared with the MACS protocol survival.**

	Our cohort	MACS protocol <sup>[52]</sup>
4 <sup>1)</sup>	10/10	17.5 %
5	6/6	15 %
6	5/5	10 %
7	3/3	10 %
8	2/2	5 %
9	1/1	5 %
Years	Patient survivals	

1) Two of this group has abandoned our treatment at the fourth year for geographic reasons. Though they are presently living, but as they are submitted to another type of treatment, we present their data at the fourth year of ours.

## DISCUSSION

**The retro-virus integrase inhibitors, full-swing HIV<sub>1</sub>-AIDS virostatics** We admit having been impressed by some authors who were considering risky to treat a viral infection with host DNA and DNA-associated enzyme attackers<sup>[29]</sup>. This was the reason why we submitted acriflavine (ACF) and hydroxy-methyl-ellipticine (HEL), which the *c-erb* B test had revealed to be integrase inhibitors<sup>[18,19]</sup>, to a repeated study of their comparative toxicology. The absence of side effects at the optimally anti-viral doses and the fact that patients treated by experts only received AZT to which their HIV<sub>1</sub> was most often resistant, conducted us to include these two integrase inhibitors into individual treatments, combined initially with AZT, and later with the discovered other virostatics.

This supposed toxicity fear can be abandoned today, as none of these two agents has, in a ten year experience, expressed one grade 2 side effect. The two other HIV<sub>1</sub> virostatic series have, on the contrary, appeared highly toxic, especially as far as late term manifestations are concerned<sup>[34-38]</sup>.

Furthermore, *in vitro* tests adapted to the screening of HIV<sub>1</sub> integrase inhibitors, have recently added to ACF

and HEL in this series of virostatics, two other and non toxic drugs, cobalamine<sup>[44]</sup> and cobalt<sup>[45]</sup>. We have confirmed, with a second application of vitamin B12, its favorable immunologic effect, not only on monocytes which concern all patients, including P (Fig 3), but also on CD4 and CTL numbers (except in P) (Fig 4-6).

Objective reviews<sup>[28]</sup> claim that HIV<sub>1</sub>-integrase inhibitors are in fact very numerous. Some are known to be available in huge quantity, and at accessible costs.

**The highly active and non toxic four drug combination regimen** The absence of adverse effects of our combinations, even of 4 drugs, is probably related to the evoked mechanism, consisting of leaving, between each application of any agent, the time for its binding sites to eliminate the drug and to repair<sup>[43]</sup>.

The significant transitory decrease of CD34 numbers observed in one patient who received 6 instead of 4 virostatics for 6 months, and of another who received minimum doses of cyclophosphamide conduct us to fear that of our four drug regimen may not be far from the superior limit of the optimum dose rank.

### Immunologic reconstitution or reactions?

The most original data concern the immunologic parameters influenced by the treatment, and their correlations not only with VL, but with the so called cofactors.

As Oldstone<sup>[50]</sup> confirmed, the types of cells which react the most and are in correlation with the initial VL decrease, are the CTL and the CD28<sup>+</sup> CTL. Their numbers are the first and the only ones to increase when the virostatics combinations include only 2 or 3 drugs. They increase much above their normal physiologic numbers when the patients receive the four drug regimen. They may however reduce their increase while the clinical and HIV<sub>1</sub> conditions are good. Their increase may in fact be related not only to HIV<sub>1</sub> attack, but also to that of some cofactors.

The other category of cells to increase, but mainly after vitamin B12 applications, are the monocytes.

**A regulatory cell paradox** What appears clearly on Fig 3 to 6 has been found at the statistical analysis to be published elsewhere; there is no significant correlation between VL reduction during its first regression phase, on one hand, and the CD4 numbers or the STL, on the other. There is a significant correlation between their positive or negative differences (data not shown).

An immunologic original pathway, exempt from regulatory cells If the immunologic "perturbations"

induced by PUVA were partially known<sup>[54]</sup>, their mechanism, at least that of the chronic and irreversible ones, are not. It is thought by some that CD4<sup>+</sup> and CTL share the same precursor<sup>[55]</sup>. The latter could be as sensitive to PUVA as differentiated T cells.

**"Cured" residual disease?** The induction of discrete and reversible HIV<sub>1</sub>-rebounds that we have called late cofactors, proves that a residual virus load, though PCR undetectable in all patients except P, persists during the asymptotes of the VL exponential curves.

The fact that neither HIV<sub>1</sub>, nor the microbial cofactors which induce these rebounds, induce relapses, suggest that they are under virostatic and/or immunologic controls. This phenomenon has been observed in chemotherapy "cured" acute lymphoid leukemia (ALL); we observed neoplastic cell rebounds<sup>[56]</sup>, which have been shown by systematic research of molecular markers, not to be exceptional<sup>[57]</sup>. Only the ALL patients submitted to active immunotherapy present long term remission curves which are true plateaux<sup>[58,59]</sup>; In fact those we have observed, only concern special HLA-phenotypes different from those which are associated with long maintenance chemotherapy<sup>[58]</sup>. Active immunotherapy for HIV<sub>1</sub>-AIDS complex might thus be proposed to CCR5<sup>[49]</sup> heterozygotic mutated subjects.

We have not used, as immunomodulator, interleukin 2, even applied at small doses. If it increases CD4<sup>+</sup> cell numbers<sup>[60]</sup>, no significant and persisting VL reducing action during the asymptotic part of its curve, has yet been observed. As far as immunotherapy is concerned, we are tempted by the use of bestatin<sup>[61]</sup>: the increase of suppressor cell numbers appears to be its best indication. It does not share with cyclophosphamide<sup>[62]</sup>, another reducer of STC numbers, the risk of stem cell reduction we discovered on patient Dlb at CD34 cell number statistical analysis.

Finally, the numerous, non toxic, RV integrase inhibitors may find their best indications in possibly necessary long "maintenance" treatment of residual disease, as most can be applied for long periods of time without toxicity, and as they may act on other retroviruses. Many, such as vitamin B12<sup>[44]</sup> and flavones<sup>[28]</sup>, act on methylation, which may be another advantage<sup>[63]</sup>.

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病毒学和免疫学随访经 4-9 年四种病毒抑制药包括整合酶抑制剂结合短程药物轮转 AIDS 治疗

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关键词 逆转录病毒科; 整合酶抑制剂; 玫瑰树碱类; 旋黄; 维生素 B12; HIV-1

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