

POSTER 522-M

Non-specific Immunotherapy with Murabutide significantly improves responses to recall and to HIV-1 antigens, in patients Naïve to Antiretroviral therapy.

X. De La Tribonniere^{1,3}, Y. Yazdanpanah¹, Y. Mouton¹ G.M. Bahr^{2,3}

¹ Infectious disease dept, Tourcoing; ² Lab. Of Molec. Immunol. of Infection, Pasteur Inst., Lille; ³ ISTAC Biotech., Lille, France

Contact :

Xavier De la Tribonnière, M.D.

((33).3.20.69.46.64
Fax : (33).3.20.69.46.15
servmalinf@ch-tourcoing.fr

ABSTRACT :

Background and method: The synthetic immunomodulator Murabutide (MB) has been reported to enhance non-specific immune defense mechanisms, to regulate cytokine synthesis, and to control HIV-1 replication in antigen-presenting cells. Following successful phase I studies in HIV-1 patients, we carried out a comparative trial on 18 patients chronically infected with HIV-1 (9 controls and 9 treated sc. with 7 mg/day of MB, 5 days/week for a period of 6 weeks) to address the potential immune benefits of non-specific immunotherapy in such patients. All patients had not received antiretrovirals and presented CD4 counts >500 cells/ μ l and viral load (VL) <30 000 copies/ml.

Results: Administration of MB was well tolerated in 7 out of 9 patients and adverse reactions were spontaneously reversible upon drug interruption. No deleterious effects on VL and CD4 counts were observed throughout the whole study period (up to 18 weeks after starting MB administrations). Improvement in functional immune assays were noted only in MB recipients. Concerning lymphoproliferative assays, we observed a general enhancement in the size of responses to 5 different recall antigens, and significant differences between the 2 groups were achieved at week 18 against CMV antigen. Also, we observed at week 18 an increase in the size of responses to HIV proteins in the MB group (against p24 and against gp160, $p < 0.01$), as well as a significant increase in the % of responders to p24 and to Nef ($p < 0.05$). Analysis of cytokine release, before and after MB treatment, revealed an increase in the capacity of PBMCs to secrete IFN-g following mitogenic stimulation. No major changes in phenotypic markers of lymphocytes and monocytes could be detected in either group.

Conclusion: This pilot study points to the capacity of MB to potentiate immune responses to recall antigens as well as the recognition of viral proteins in HIV-1 patients naïve to antiretroviral therapy. Moreover, these results open new avenues for extending the use of non-specific immunotherapy to different settings including structured treatment interruptions.

Introduction

It is well documented that quantitative and qualitative alterations of both arms of the immune system occur during HIV infection. In addition, these defects are not completely restored under highly active antiretroviral therapy (HAART) despite a controlled viral replication in HAART-treated patients. Furthermore, one of the greatest challenges in HIV field nowadays is to avoid to initiate HAART too early and for too long because of the numerous adverse reactions and the high cost.

One of the strategies evaluated to reduce the HAART weight and to tend to restore immune recognition of HIV antigens is Structured Therapeutic Interruptions (STI). It appears that the “vaccination effect” of STI is quite efficient in acutely HIV-1-infected patients, but not in those chronically infected. There is a general consensus on the need for immune intervention to correct certain deficits in the innate arm of the immune system which would then allow vigorous and long-lasting responses to HIV antigens during the STI windows. In that context, non-specific immunotherapy could potentially represent a breakthrough. However, it should not take place at the expense of increased viral load or decreased CD4⁺ T cell counts.

The immunomodulator Murabutide could be a good candidate to use during STI. In vitro, it enhances the secretion of T-helper 1 cytokines and inhibits HIV replication in different cell types. Furthermore, results of the RESTIMUR trial, presented at ICAAC 2001, were encouraging. RESTIMUR was a pilot randomised clinical trial on 42 HAART-treated patients presenting a limited restoration of their altered immune system. In the treated group, we observed a significant increase in CD4⁺ cell counts and a trend towards a reduced viral load (pVL). An improvement in functional immune assays, including a higher percentage of responders to recall as well as to HIV antigens, was also observed in Murabutide recipients but not in the control group.

In a perspective to propose immunotherapy with Murabutide during STI regimens, we have to assert, in HIV infected patients naive to HAART, the tolerance of the immunomodulator, and its effect on viral loads as well as on immunological parameters.

Objectives

To evaluate the clinical tolerance and the biological activity of Murabutide in HIV- infected patients, naive to HAART, with relatively maintained immune status and controlled viral loads.

Patients and methods

Study design

Novimur was a tridentric, pilot, open-labelled comparative phase I/IIa trial (Northern France reference centre for HIV disease at Tourcoing hospital, Valenciennes and Amiens hospitals). Ethical agreement was obtained from the French Independent Ethics Committee (CCPPRB) in Lille, France.

Study population included :

9 patients in treated and 9 in control group (**table 1**)

- HIV seropositive adults
- Contamination with HIV dating for more than 6 months
- CD4 cell count > 500/mm³
- HIV-1 pVL < 30 000 copies/ml
- Patient naive to antiretrovirals

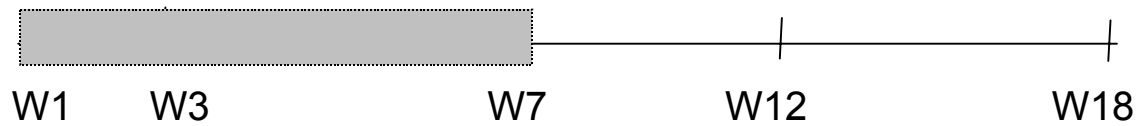
End points measures

* Principal end point : clinical and biological safety

* Secondary end point : changes in the functionality of the immune system

Therapeutic regimen and follow-up

Murabutide treatment : 7 mg/day, 5 days/week, 6 consecutive weeks (i.e., 30 injections)



W_x : week in which patients were followed up

Results conclusion

- Clinical Tolerance (table 2)

* Murabutide was well clinically tolerated in 67% of the patients (grade I). Only 1 patient presented grade II and 2 patients presented grade III adverse events. No grade IV toxicity was observed.

* Only one patient out of 9 had to prematurely discontinue the treatment (after 5 weeks) because of a grade III side effect. In addition, the dose of Murabutide did not have to be lowered in any case.

* The grade I/II side effects observed could be divided into 2 categories :

- flu-like syndrome, which rapidly disappeared within few days.
- events that appeared with the repeated injections from the second week onward and which got progressively worse with time. This category mainly included monoarthralgia, increase in the size of adenopathies and pain at the injection site.

⑩ In one case, we observed a grade III increase in the size of axillar and cervical adenopathies. A surgical biopsy has been performed and the histopathological result showed a common lymphoid hyperplasia. Spontaneous resolution was noted after treatment cessation.

- Good biological tolerance (table 2).

- Clinical surrogate markers (figure 1)

- no increase in HIV pVL
- no decrease in CD4 count, in % CD4 cells or in CD4/CD8 ratio
- stability over time of other surrogate markers: CD8 count, % CD8 cells
- a significant increase in platelet counts in the Murabutide treated group (intragroup comparison), with a mean increase of 14,000 platelets/mm³ at W3 and 18,000 at W12, leading to a 7 to 9 % increase in the total number of platelets on average at W3 and W12 compared to baseline.

- Immunological analyses

- * the hallmark of the effects induced by Murabutide consisted of :
 - . enhanced cellular responses to recall antigens (**figure 2**).
 - . enhanced cellular responses to HIV proteins (**figure 3**).
- * no changes in the serum cytokine profiles or in the in vitro PHA-induced cytokines and chemokines levels, unless a significant increase at W18 in the capacity of PBMCs to secrete IFN- γ following mitogenic stimulation.
- * no changes in the expression on lymphocytes and/or monocytes of the following immunophenotypic markers CD62L, CD45RA, CD95, CD28, CD38, HLA-DR, CD86, CCR5, and CXCR4.

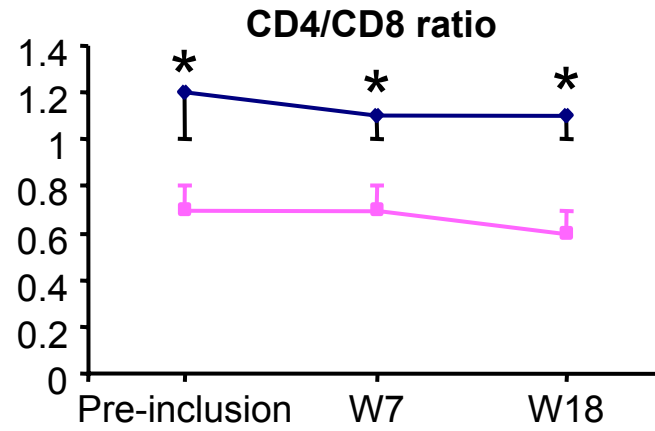
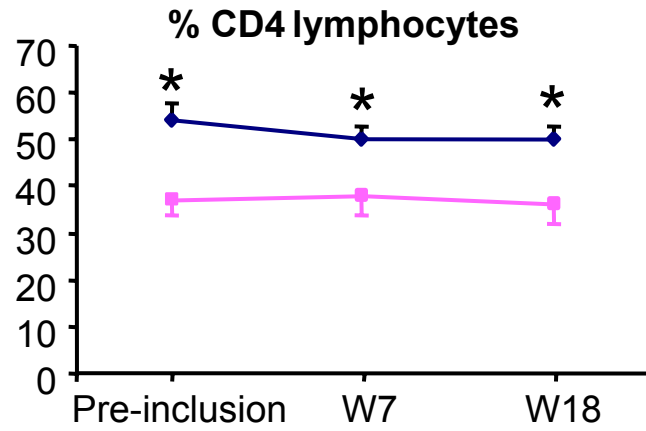
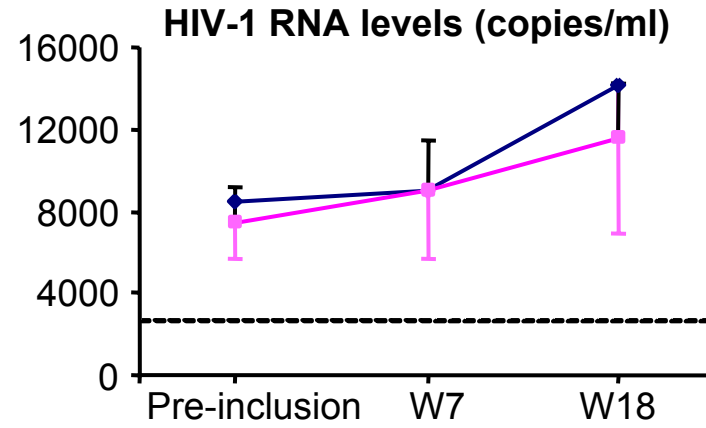
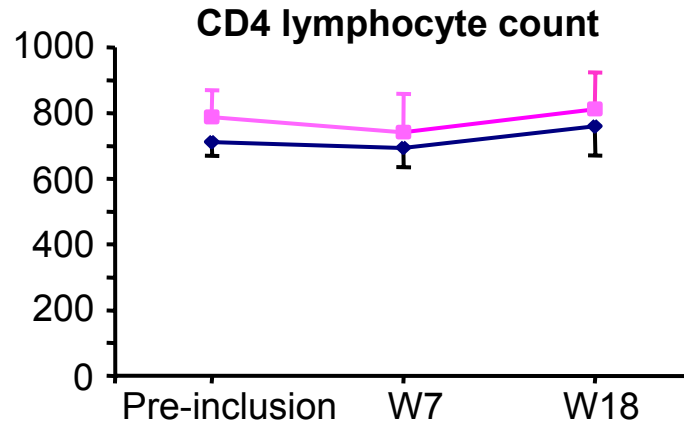
Discussion and conclusion

- The clinical and biological tolerance of 6 weeks administration of Murabutide into HIV-1 infected patients, naïve to HAART, was quite acceptable.
- In contrast to the reported effects with other endogenous immunomodulators, immunotherapy with Murabutide in naive patients neither increased viral loads nor decreased CD4 counts.
- A 6 weeks immunotherapy with Murabutide induced tangible changes in cell-mediated immune responses. A general improvement in the lymphoproliferative responses was obtained against a battery of recall antigens. More importantly, the responses to different HIV proteins were significantly increased 12 weeks after the period of immunotherapy. These results substantiate the hypotheses that correction of deficits in innate immunity by non specific immunotherapy could simultaneously induce recovery of specific responses to the virus.
- These data open new avenues for extending the use of non-specific immunotherapy with Murabutide to different settings including STI. The potential association of Murabutide with the therapeutic cytokines, IL-2 and IFN- γ , may prove to be a highly efficacious approach for the immunotherapy of HIV infection.

Références

- 1- Bahr GM, De La Tribonnière X, Mouton Y. Novel Approach of Non Specific Immunotherapy, as Adjunct to HAART, in HIV-1 patients with Poor Immunological Recovery. *ICAAC 2001, Chicago, Abst. 1960.*
- 2- Bahr GM, Cocude C, Billaut-Mulot O, Truong MJ, Capron C. The cytokine inducer Murabutide inhibits the expression of a novel cellular RNA helicase necessary for HIV replication. *International cytokine society 2001; abst 202*
- 3- Amiel C, De la Tribonnière X, Vidal V, Bahr GM, Mouton Y. First phase I/II clinical trials with a new synthetic immunomodulator, Murabutide, in HIV infected patients under combined antiretroviral therapy. submitted.
- 4- Darcissac CA, Truong MJ, Dewulf J, Mouton Y, Capron A, Bahr GM. The synthetic Immunomodulator Murabutide controls human immunodeficiency virus type I replication at multiple levels in macrophages and dendritic cells. *J Virol 2000, 74: 7794-7802*
- 5- Bahr GM, Darcissac E, Castéran N, Amiel C, Cocude C, Truong MJ, Dewulf J, Capron A, Mouton Y. Selective regulation of human immunodeficiency virus-infected CD4+ lymphocytes by a synthetic immunomodulator leads to potent virus suppression in vitro and in hu-PBL-SCID mice : implications for the immunotherapy of HIV diseases. *J Virol 2001, 75: 6941-52*
- 6- Vidal V, Dewulf J, Bahr GM. Enhanced maturation and functional capacity of monocyte-derived immature dendritic cells by synthetic immunomodulator Murabutide. *Immunol 2001, 103: 479-87*
- 7- Vidal V, Castéran N, Riendeau CJ, Kornfeld H, Darcissac E, Capron A, Bahr GM. Macrophage stimulation with Murabutide, an HIV-suppressive muramylpeptide derivate, selectively activates extracellular signal-regulated kinases 1 and 2, C/EBP and STAT1 : role of CD14 and toll-like receptors 2 and 4. *Eur J Immunol 2001 , 31 : 1962-71*
- 8- Darcissac ECA, Vidal V, Guillaume M, Thebault J-J, Bahr GM. Clinical tolerance and profile of cytokine induction in healthy volunteers following the subcutaneous administration of interferon - γ and the immunomodulator Murabutide. *J. Interferon Cytokines Res, 2001, 27: 655-61*

Figure 1

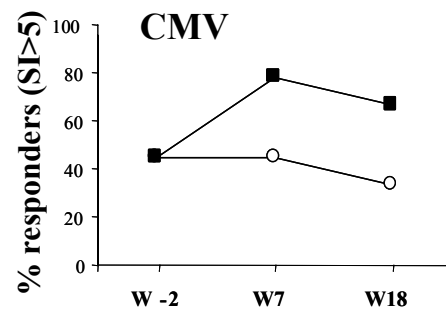
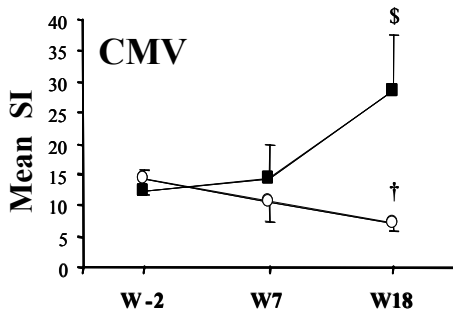
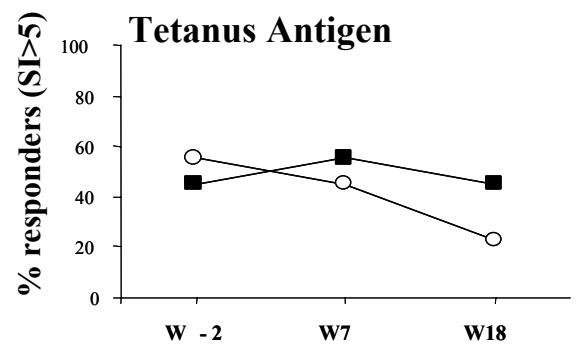
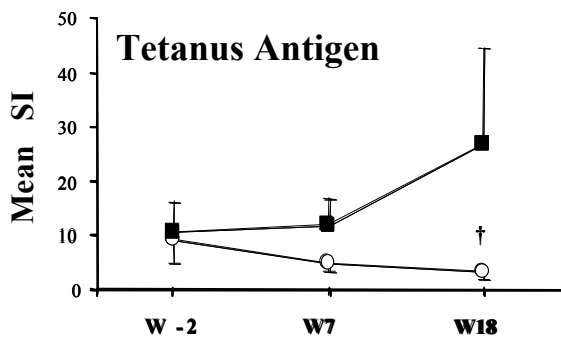
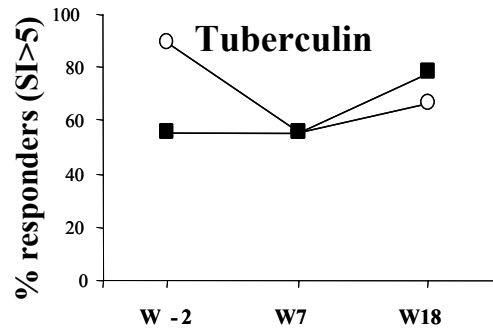
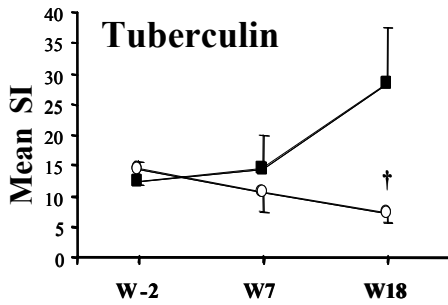
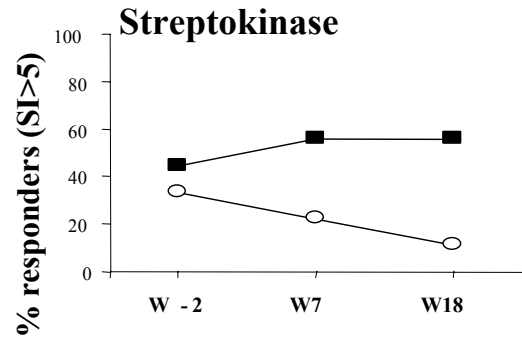
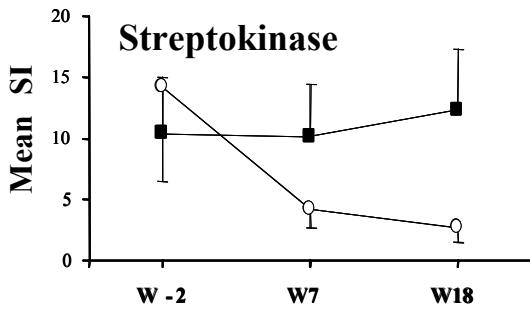
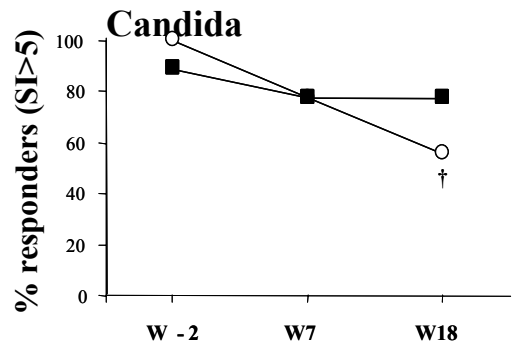
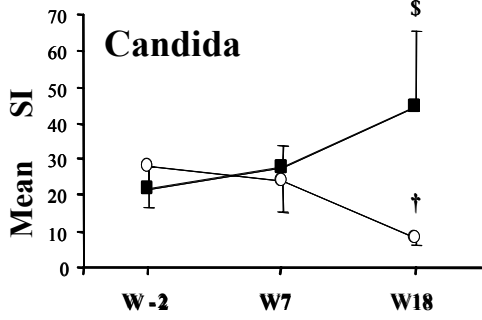


◆ Control group

■ Murabutide-treated group

* P < 0.05

Figure 2



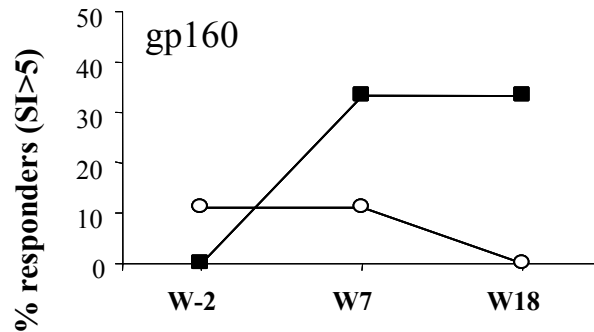
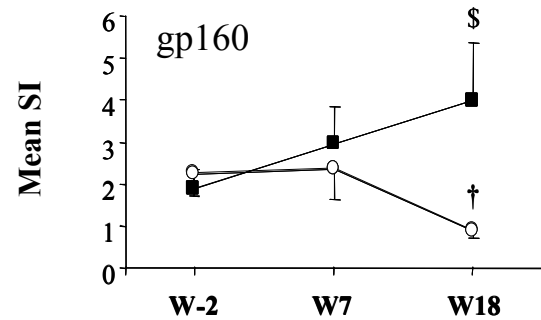
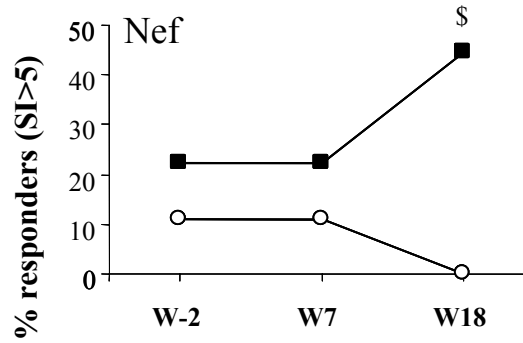
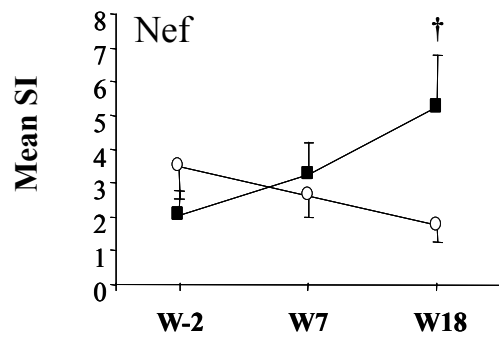
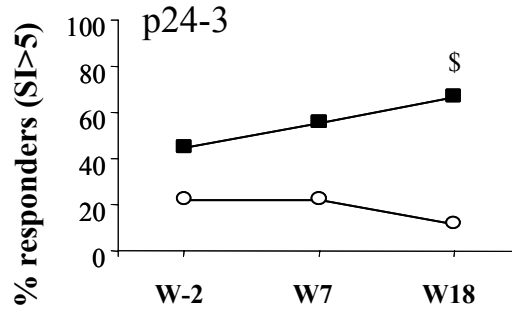
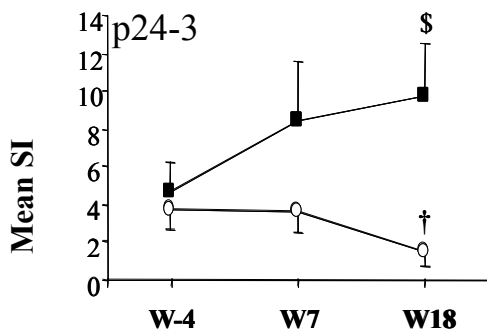
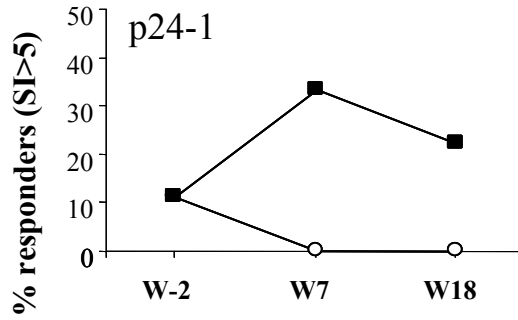
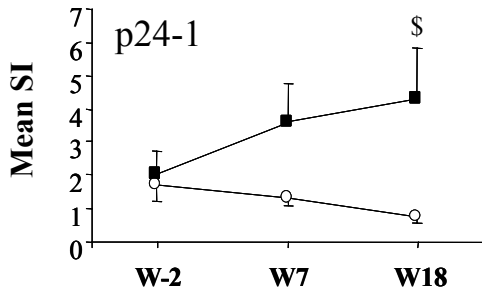
○ Control group

■ Murabutide-treated

^{\$} vs. control group (Fisher's Exact Test)

[†] vs. values at W-2 (Fisher's Exact Test)

Figure 3



○ Control group \$ vs. Control group (Fisher's Exact Test)
 ■ Murabutide - Treated † vs. Values at W - 2 (Fisher's Exact Test)

Table 1 - Characteristics of the populations under study

Characteristics	Treated group (n=9)	Control group (n=9)
Sex (M :F)	5 :4	6 :3
Age (years, mean \pm SEM)	36 \pm 2	37 \pm 3
HIV risk factors		
Homosexual	4	4
Heterosexual	4	5
Intravenous drug use	0	0
Unknown	1	0
Time since documentation of HIV-1 seropositivity (years, mean \pm SEM)	4 \pm 1	4 \pm 1
CDC category	4	7
A1	1	1
A2	4	0
B1	0	1
B2		

Table 2 : Number of patients in the treated group presenting side effects probably, possibly or doubtfully related to Murabutide

Clinical side effects	Grade I	Grade II	Grade III	TOTAL
Flu like syndrome/headache	2	1		3
Asthenia	3			3
Arthralgia/back pain	5		1	6
Myalgia	4			4
Increase of adenopathy	4		1	5
Pain at injection point	4	1		5
Lateral intercostal pain	1			1
Digestive disorder ^a	2	1		3
Thymic disorder ^b	3			3
Other effects ^c	4			4
Biological side effects				
Neutropenia	1	1		2
Increase in ALAT level	1			1
Increase in lipase level	3	2		5
Increase in triglyceridemia	1			1
Increase in CPK level		1	1	2
Total of number of patients presenting clinical side effects ^d	6	1	2	9

^a : nausea, epigastralgia, dysphagia

^b : irritability, depression

^c : impression of swollen throat, difficulty in falling asleep, heaviness of legs, numbness of fingers, increase of thirst, increase of pruritis

^d : for each patient, only the highest grade of side effect was taken into account.