blood

Prepublished online October 13, 2011;
doi:10.1182/blood-2011-04-349910

Safety of long-term treatment of HAM/TSP patients with valproic acid

Stéphane Olindo, Gildas Belrose, Nicolas Gillet, Sabrina Rodriguez, Mathieu Boxus, Olivier Verlaeten, Becca Asquith, Charles Bangham, Aïssatou Signaté, Didier Smadja, Agnès Lezin, Raymond Césaire and Luc Willems

Blood (print ISSN 0006-4971, online ISSN 1528-0020), is published weekly by the American Society of Hematology, 2021 L St, NW, Suite 900, Washington DC 20036.

Safety of long-term treatment of HAM/TSP patients with valproic acid

Stéphane Olindo [1], Gildas Belrose [2], Nicolas Gillet [3] Sabrina Rodriguez [3], Mathieu Boxus [3], Olivier Verlaeten [3], Becca Asquith [4], Charles Bangham [4], Aïssatou Signaté [1], Didier Smadja [1], Agnès Lezin [2], Raymond Césaire [2] and Luc Willems [3,5]

[1] Service de Neurologie and JE 2503, Centre Hospitalier Universitaire de Fort-de-France, 97200 Fort-de-France, Martinique, France

[2] Laboratoire de Virologie-Immunologie and JE 2503, Centre Hospitalier Universitaire de Fort-de-France, 97200 Fort-de-France, Martinique, France

[3] Molecular and cellular biology, Gembloux Agricultural University, 5030 Gembloux, Belgium and Interdisciplinary Cluster for Applied Genoproteomics (GIGA), University of Liège (ULg), Belgium

[4] Department of Immunology, Wright-Fleming Institute, Imperial College London, St Mary's campus, London, UK

[5] Corresponding author: Luc Willems, Molecular and Cellular Epigenetics, Interdisciplinary Cluster for Applied Genoproteomics (GIGA) of University of Liège (ULg), avenue de l'Hôpital B34 Sart-Tilman, 4000 Liège – Belgium.

Tel: 32-(0)-4-3662978 Fax: 32-(0)-81-613888 email: luc.willems@ulg.ac.be

Short title: HAM/TSP treatment with VPA

ABSTRACT

HTLV-Associated Myelopathy/Tropical Spastic Paraparesis (HAM/TSP) is a neurodegenerative disease of the central nervous system induced by Human T-lymphotropic virus type 1 (HTLV-1). As a potential therapeutic approach, we previously suggested reducing the proviral load (PVL) by modulating lysine deacetylase activity using valproic acid (VPA) and exposing virus-positive cells to the host immune response. We conducted a single-center, two-year open-label trial, with 19 HAM/TSP volunteers treated with oral VPA. PVL, CD38/HLA-DR expression and CD8+ lysis efficiency were not significantly affected by VPA. Mean scores of HAM/TSP disability did not differ between baseline and final visit. Walking Time Test (WTT) increased significantly (>20%) in 3 patients and was in keeping with minor VPA side effects (drowsiness and tremor). WTT improved rapidly after VPA discontinuation. We conclude that long term treatment with VPA is safe in HAM/TSP.

INTRODUCTION

Among 15-20 million HTLV-1 infected individuals worldwide, about 5% will develop one of the two major HTLV-1 associated diseases, HAM/TSP and Adult T-cell Leukemia (ATL) [1]. HAM/TSP is a neuroinflammatory disease of the central nervous system associated with perivascular and parenchymal infiltration of HTLV-1-infected T-cells and activated cytotoxic T lymphocytes (CTL). Collateral damage results from release of proinflammatory cytokines such as IFN-γ and TNF-α by invading CD4+ and CD8+ infected cells and HTLV-1-specific T-lymphocytes. The immune response controlling infection may therefore also become detrimental and ultimately participate in mediating HAM/TSP progression [2]. A key determinant of the development of HAM/TSP is the level of HTLV-1-infected cell burden, the proviral load (PVL) in peripheral blood mononuclear cells (PBMCs) being 5 to 20-fold higher in patients with HAM/TSP than in asymptomatic carriers [3]. A PVL $>10^5$ HTLV copies per million PBMCs constitutes a predictor of rapid progression from disease onset to Disability Status Scale (DSS) 8 (wheelchair confinement) [4].

In absence of satisfactory therapy and based on preclinical trials performed in an animal model [5], we proposed a strategy designed to activate viral gene expression in order to expose virus-positive cells to the host immune response. We used VPA, a lysine deacetylase inhibitor that modulates chromatin condensation to alter the pattern of gene expression. We demonstrated that in ex vivo cultures of HAM/TSP PBMCs, VPA induces hyperacetylation, activates expression of the viral core protein p19 and triggers apoptosis of CD4+ and CD8+ T lymphocytes [6]. Real time quantification performed in the Virology-Immunology reference laboratory of Fort-de-France indicated that administration of VPA to HAM/TSP patients led to a decrease in their PVL a few weeks after initiation of treatment [6].

We conducted a single-center, two-year open-label trial, with 19 HAM/TSP volunteers treated with oral doses of VPA. Objectives were to characterize ongoing mechanisms, assess clinical safety and evaluate biological response to VPA treatment. The following questions were addressed:

1. Is the CTL efficiency preserved and not exacerbated?
2. How do the PVL evolve in the long term?
3. What is the condition of treated patients?

METHODS

VPA given orally (20 mg/Kg/day) was planned to be given for 24 months when the trial started. Clinical assessment was performed every 3 months by the same observer. DSS was used as a measure of global neurological disability, 30-meter Walking Time Test (WTT) quantified gait performance, manual muscle score and modified Ashworth score were applied to 9 muscle groups in both legs and urinary disability was assessed by a short questionnaire. Overall analysis of neurological disability test was performed based on observed cases and also using the last observation carried forward (LOCF) method. Worsening of neurological disability was defined as: significant mean scores or time differences between M0 and M24 or M0 and the last assessment visit before treatment discontinuation and individually by the increase DSS score of 1 or 0.5 point as regard as baseline DSS (<5.5 or ≥ 5.5 respectively) or a WTT variation rate >20%. The study was approved by the Local and Regional Research Ethics Committee and all procedures were carried out in accordance with the Declaration of Helsinki.

The efficiency of CD8+ cell mediated lysis before and after VPA treatment was performed as described by Mosley et al [8]. The proportions of CD4+ or CD8+ cells expressing CD38 or

HLA-DR were determined by flow cytometry using the MultiTEST assay from Becton Dickinson.

RESULTS AND DISCUSSION

A major concern with VPA is that it may impair the anti-viral immune response. Indeed, the rate of CD8+ cell-mediated lysis of Tax expressing cells ex vivo is halved in short term cultures complemented with a 5mM dose of VPA [8]. Although this concentration is indeed above the levels that can be achieved in patients (estimated about 1-2 mM), the risk of long term treatment with lower doses cannot be predicted. Since the CTL response is an important factor in the immune control of HTLV-1 infection [2], we addressed this question directly by measuring the efficiency of CD8+ cell mediated lysis. The cytotoxic response was evaluated by an assay in which the number of Tax+ cells surviving autologous CD8+ killing is measured by FACS after overnight culture [9]. The percentages of surviving Tax+ CD4+ cells were then plotted against the proportion of CD8+ cells present (duplicate assays are shown in Figure 1A: ■ and □). Lysis efficiency, which was estimated by fitting a mathematical model to these data [9], was calculated and expressed as the proportion of Tax-expressing CD4+ cells killed per CD8+ cell per day (Figure 1B). To assess whether these variations were within the normal range observed in untreated patients, the initial rate of lysis efficiency was plotted against the median absolute change in lysis efficiency between consecutive time points. Lysis efficiency variations observed during VPA treatment (□) were not significantly different from those in untreated patients (Figure 1C) (♦) (P=0.072, Wilcoxon-Mann-Whitney 2-tailed test). We conclude that CD8+ cell-mediated lysis efficiency fluctuates throughout treatment but remains within the normal range, indicating that the subject's CTL immune response against HTLV-1 is not significantly suppressed by VPA. Likewise, the CTL response is not

significantly exacerbated and must not be a concern regarding HAM/TSP progression.

To support this conclusion, we next assessed T cell activation as defined by expression of CD38 and HLA-DR. PBMCs from 5 patients before and 3 months after initiation of VPA treatment were analyzed by FACS (illustrated for patient #4 on the plots of figure 1D and recapitulated for 5 patients on Figure 1E). It appeared that there is no significant difference in the proportions of CD4+ or CD8+ cells expressing CD38 and HLA-DR.

The initial objective of VPA treatment in HAM/TSP patients was to permanently reduce the PVL with the aim of attenuating collateral damages to the central nervous system. However, long term administration of VPA did not achieve this goal. The PVL determined by real- time PCR [10] after 12 and 24 months or the last quantification before treatment discontinuation was similar to before treatment (Figure 2A). Based on LOCF analysis, no significant differences were found in mean neurological scores and WTT (Figure 2B). No patient's DSS score increased significantly over the study. Eight of 19 patients stopped VPA treatment before M24 (Figure 2C). Three patients were withdrawn because of a significant increase in the rate of variation of WTT. WTT improved rapidly after treatment discontinuation. Gait impairment worsening was probably related to severe drowsiness and tremor side effects experienced by 3 patients. The main clinical side effects of VPA were drowsiness (52%), tremor (47%), digestive symptoms (37%), vertigo (26%), and alopecia (10%) and their frequencies tended to decrease over the trial course. No significant biological side effect was documented. Walking deterioration in few patients may be associated with VPA side effects and is quickly reversible after treatment discontinuation.

In summary, we have shown that CTL response is preserved upon VPA treatment of HAM/TSP patients. Although clinical symptoms are not improved, our data clearly indicates that long term treatment with VPA is safe. This report is important in view of the recent

evidence in STLV-1 infected baboons in which combined treatment with VPA and azidothymidine efficiently decreases PVL [11]. If effective in human, long term administration of VPA and AZT may reduce the risk of HAM/TSP. Safety is also crucial for maintenance therapy of acute ATL where molecular clearance was observed with VPA combined with AZT+INF–α [12].

COMPETING INTERESTS

The authors have no competing interests to declare.

AUTHORS' CONTRIBUTIONS

S.O., A.S., D.S. performed clinical evaluation of VPA treatment;

G.B., O.V. and A.L. measured proviral loads;

N.G. and C.B. performed CTL lysis assay;

M.B. performed the FACS analysis of CD38 HLA-DR;

B.A. modeled data;

S.O., A.L., S.R., R.C. and L.W. designed, coordinated and supervised studies;

S.O. and L.W. wrote the paper

ACKNOWLEDGEMENTS

This work was supported by the ''Fonds National de la Recherche Scientifique'' (FNRS), the Télévie, the Belgian Foundation against Cancer, the Sixth Research Framework Programme of the European Union (project INCA LSHC-CT-2005-018704), the ''Neoangio'' excellence program and the ''Partenariat Public Prive´'' PPP INCA of the ''Direction générale des Technologies, de la Recherche et de l'Energie/DG06'' of the Walloon government, the ''Action de Recherche Concertée Glyvir'' of the ''Communauté française de Belgique'', the "Fonds spéciaux pour la Recherche" of the University of Liège (ULg), the Synbiofor program of GxABT (ULg), the ''Centre anticancéreux près ULg'' (CAC) and the ''Plan Cancer'' of the ''Service Public Fédéral''. MB, NG and SR (Postdoctoral Researchers)

and LW (Research Director) are members of the FRS-FNRS.

REFERENCES

1. Matsuoka M, Jeang KT: Human T-cell leukaemia virus type 1 (HTLV-1) infectivity and cellular transformation. *Nat Rev Cancer*. 2007;7(4):270-280.
2. Asquith B, Bangham CR 1: How does HTLV-I persist despite a strong cell-mediated immune response? *Trends Immunol*. 2008;29(1):4-11.
3. Nagai M, Usuku K, Matsumoto W et al. Analysis of HTLV-I proviral load in 202 HAM/TSP patients and 243 asymptomatic HTLV-I carriers: high proviral load strongly predisposes to HAM/TSP. *J Neurovirol*. 1998;4(6):586-593.
4. Olindo S, Cabre P, Lezin A et al. Natural history of human T-lymphotropic virus 1-associated myelopathy: a 14-year follow-up study. *Arch Neurol*. 2006;63(11):1560-1566.
5. Achachi A, Florins A, Gillet N et al. Valproate activates bovine leukemia virus gene expression, triggers apoptosis, and induces leukemia/lymphoma regression in vivo. *Proc Natl Acad Sci U S A*. 2005;102(29):10309-10314.
6. Lezin A, Gillet N, Olindo S et al. Histone deacetylase mediated transcriptional activation reduces proviral loads in HTLV-1-associated myelopathy/tropical spastic paraparesis patients. *Blood*. 2007;110(10):3722-8.
7. Defoiche J, Debacq C, Asquith B, Zhang Y, Burny A, Bron D, Lagneaux L, Macallan D, Willems L. Reduction of B cell turnover in chronic lymphocytic leukaemia. *Br J Haematol*. 2008;143(2):240-7.
8. Mosley AJ, Meekings KN, McCarthy C, Shepherd D, Cerundolo V, Mazitschek R, Tanaka Y, Taylor GP, Bangham CR. Histone deacetylase inhibitors increase virus

gene expression but decrease CD8+ cell antiviral function in HTLV-1 infection. *Blood*. 2006;108(12):3801-7.

9. Asquith B, Mosley AJ, Barfield A et al. A functional CD8+ cell assay reveals individual variation in CD8+ cell antiviral efficacy and explains differences in human T-lymphotropic virus type 1 proviral load. *J Gen Virol*. 2005;86(Pt 5):1515-1523.
10. Dehee A, Cesaire R, Desire N et al. Quantitation of HTLV-I proviral load by a TaqMan real-time PCR assay. *J Virol Methods*. 2002;102(1-2):37-51.
11. Afonso PV, Mekaouche M, Mortreux F, Toulza F, Moriceau A, Wattel E, Gessain A, Bangham CR, Dubreuil G, Plumelle Y, Hermine O, Estaquier J, Mahieux R. Highly active antiretroviral treatment against STLV-1 infection combining reverse transcriptase and HDAC inhibitors. *Blood*. 2010;116(19):3802-8.
12. Ramos J, Toomey N, Diaz L, Ruiz P, Barber G, Harrington W. Targeting HTLV-I latency in Adult T-cell Leukemia/Lymphoma. *Retrovirology* 2011;8:A48.

FIGURE LEGENDS

Figure 1: CTL lysis efficiency and CD38/HLA-DR expression in HAM/TSP subjects treated with VPA

A. Before and after VPA treatment (e.g. days -6, +29 and +42 are illustrated), the rate of CD8+ cell-mediated lysis of HTLV-1-infected cells was estimated as described in reference [9]. CD8+ lymphocytes were selected by MACS and titrated back into the CD8-depleted fraction at different ratios. Reconstituted cell populations were co-cultivated at 37°C for 18 hours, fixed and analyzed by FACS for Tax, CD4 and CD8 expression. The proportion of Tax+ CD4+ cells surviving co-culture was plotted against the proportion of CD8+ cells present. Two independent experiments were performed with 3 HAM/TSP patients (#1, #2 and #3).

B. A previously described mathematical model was then used to analyze the data [9]. The model describes the onset of Tax expression in CD4+ cells and the lysis of Tax+CD4+ cells by CD8+ cells. The model was solved analytically and then fitted to the data using non-linear regression. The rate of lysis of CD4+ Tax+ cells "CD8+ cell lysis efficiency", is estimated. CD8+ cell lysis efficiency (expressed as the proportion of Tax-expressing CD4+ cells killed per CD8+ cell per day) was calculated for each HAM/TSP patient tested. All assays were done in duplicate and the results are presented as the mean CD8+ cell lytic efficiency. Indicated values result from experiments performed in duplicate at days -6, +10, +15, +29, +35, +42 and +56.

C. The median absolute change in lysis efficiency between consecutive time points was plotted against initial rate of lysis efficiency for control patients (♦) and VPA treated patients (□).

D. Expression of CD38 and HLA-DR in CD4+ cells before (at month -1: upper plots) and after (at month +3: lower plots) initiation of VPA treatment. PBMCs were labeled with the MultiTEST CD4 FITC/CD38 PE/CD3 PerCP/Anti–HLA-DR APC and analyzed with a FACS Aria (Becton Dickinson). After gating of CD3+ cells (left panels: PerCP), the percentages of FITC-labeled CD4+ cells expressing either CD38 (middle plots: PE) or HLA-DR (right panels: APC) were determined.

E. FACS analysis of PBMCs isolated from 5 patients before (month -1: M-1) and after (at 3 months: M3) initiation of VPA treatment: percentages of CD4+ or CD8+ (CD4+ CD3-) cells expressing CD38 or HLA-DR.

Figure 2: Clinical evaluation of VPA treatment

A. Proviral load in 19 HAM/TSP patients among whom 8 discontinued the treatment before the end of the study. Genomic DNA was extracted at month 0 (M0), 12 (M12) and 21 (M21) using Trizol (Sigma-Aldrich) and HTLV-1 proviral load was quantified using a real-time TaqMan PCR method, as described previously [10]. The value for the HTLV-1 proviral load was reported as the [(HTLV-1 average copy number)/ (albumin average copy number)] x 2 x 10^3 and expressed as the number of HTLV-1 copies per 10^3 cells.

B. Baseline demographic characteristics of the patients and comparison of mean neurological scores between M0 and M24 or last visit before treatment discontinuation.

C. Patients withdrawn over the 24 months of the study.

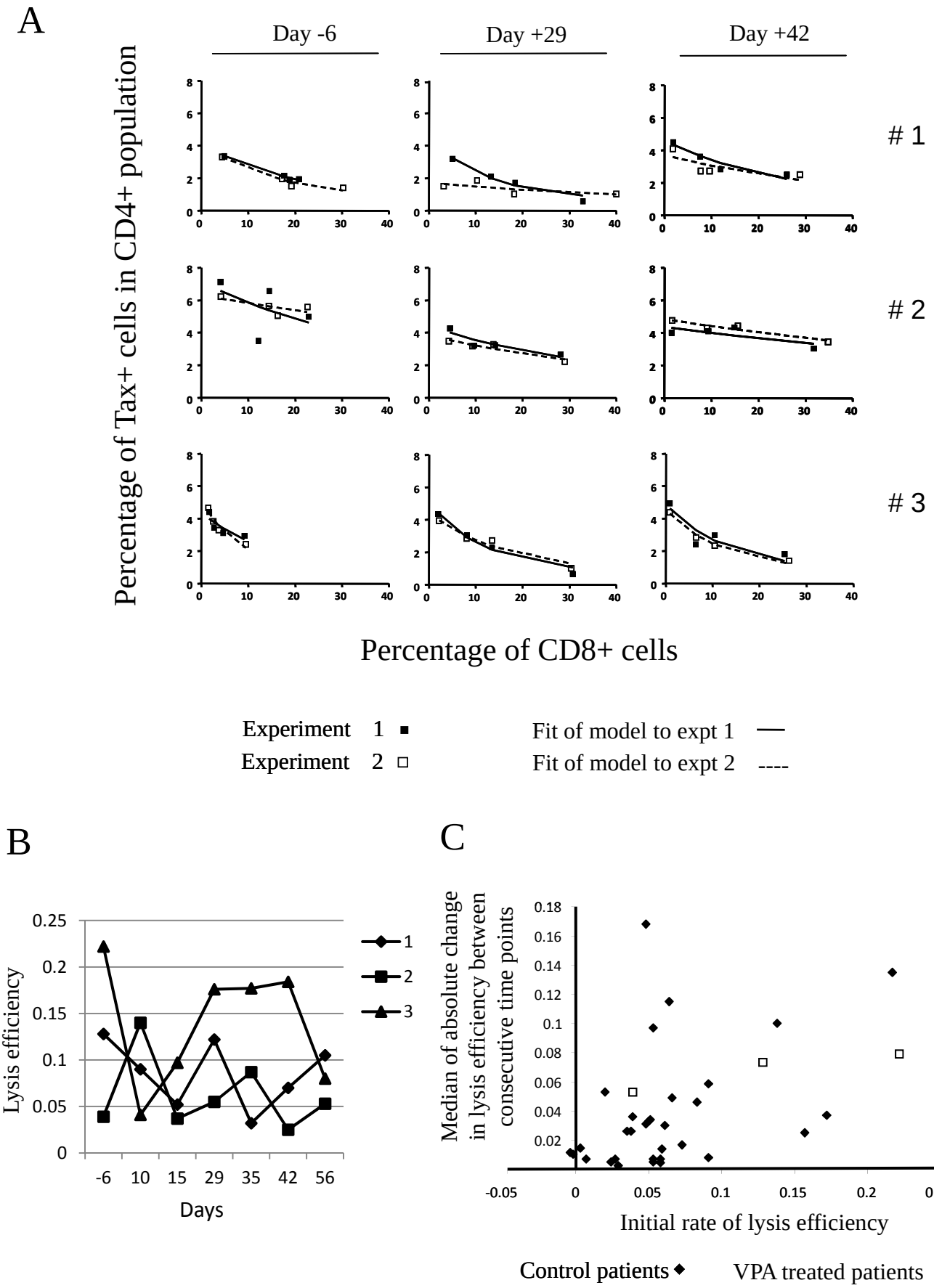
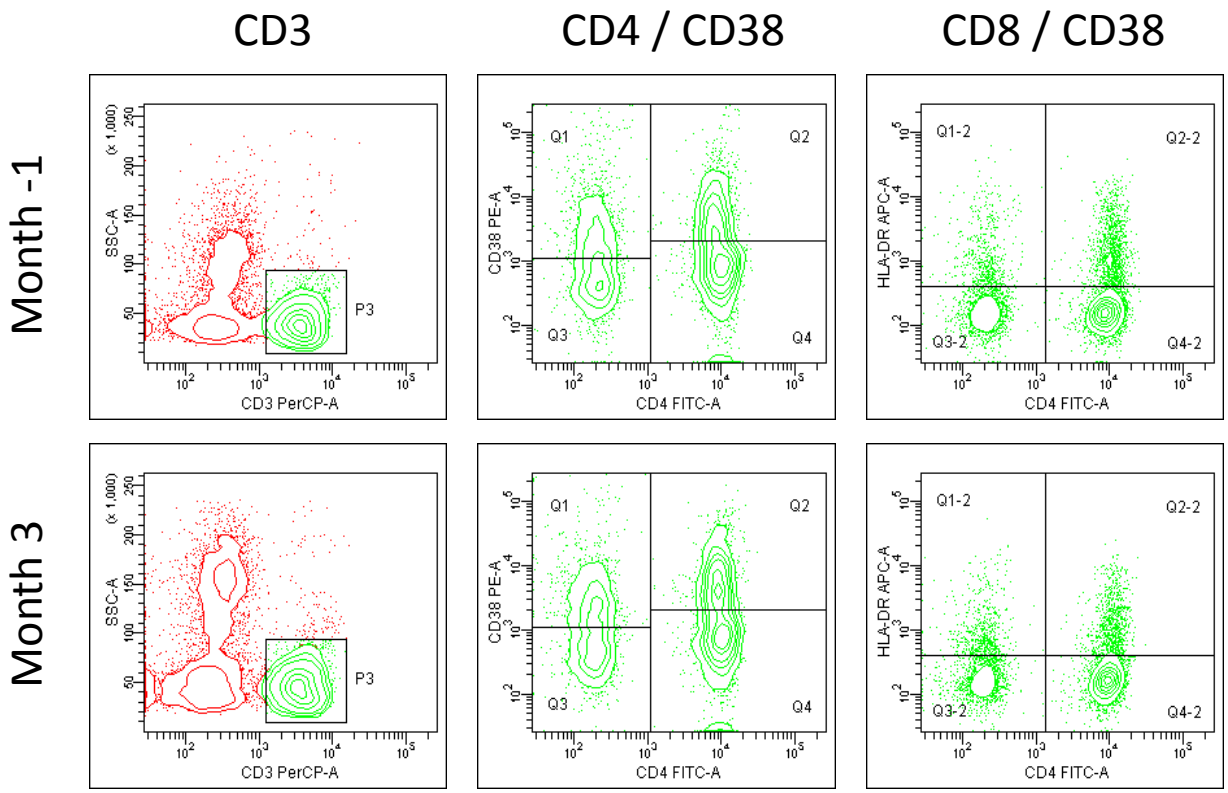


Figure 1 A and B

D



E

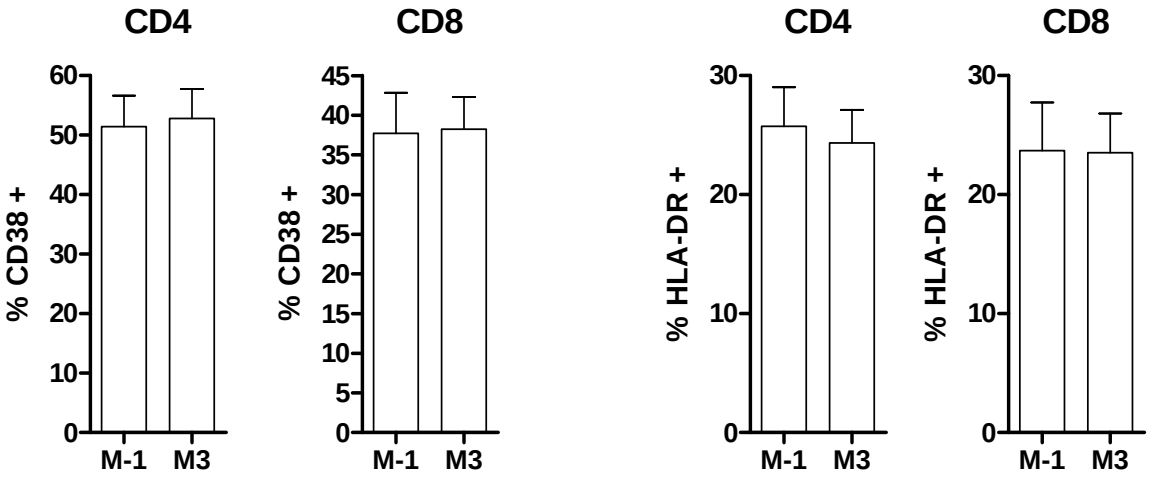
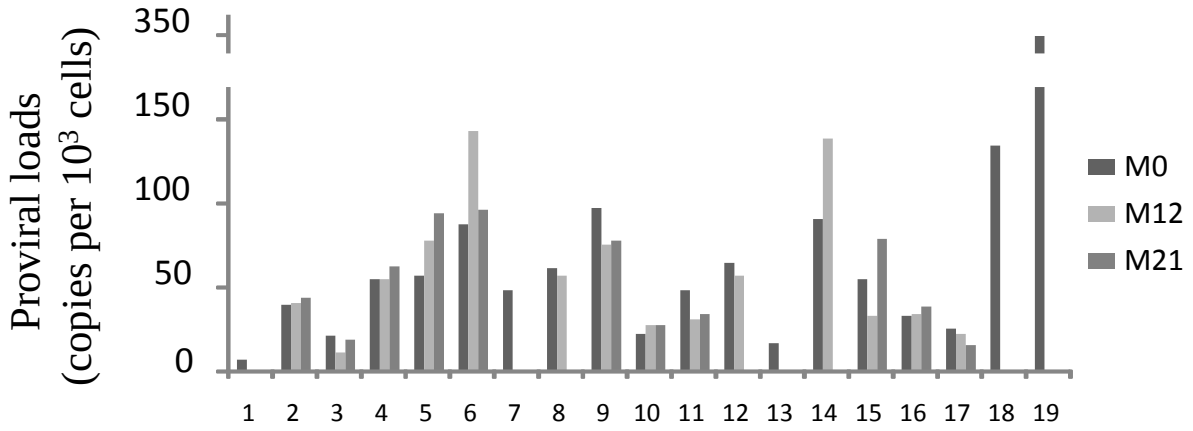


Figure 1 C and D

A



B

	M0	M24 or Last Visit	p
Age at Onset Mean years ± SD (ranges)	53.4±12 *(35-69)*		
Disease Duration Mean years ± SD (ranges)	10±4.7 *(1-18)*		
Female/Male	16/3		
DSS Mean score ± SD (ranges)	6.1±0.7 *(4-7)*	6.1±0.7 *(4-7)*	0.33
Walking Time Test Mean seconds ± SD (ranges)	76.1±45 *(28-180)*	88.8±64 *(26-240)*	0.24
Manual Muscle Test Mean score ± SD (ranges)	61.7±13 *(32-86)*	59.6±10.5 *(34-73)*	0.07
Modified Ashworth Test Mean score ± SD (ranges)	2.6±3.2 *(0-8)*	3.4±3.4 *(0-10)*	0.62
Urinary Disability Measure Mean score ± SD (ranges)	7.5±3 *(4-12)*	7.3±2.8 *(3-12)*	0.99

Figure 2 A and B

C

Withdrawn Patients between M0 and M24 (n=8)		
Month	Withdrawn cause	n
M3	Lost Interest and lost to follow-up	1
M3	Lost Interest	1
M3	Significant worsening of WTT	1
M7	Significant worsening of WTT	1
M12	Significant worsening of WTT	1
M13	Lost Interest	1
M14	Death in relation with brain tumour	1
M16	Lost Interest	1

Figure 2C