**Journal of NeuroVirology,** 14: 73–77, 2008

ISSN: 1355-0284 print / 1538-2443 online
DOI: 10.1080/13550280701801107

informa
healthcare

*Short Communication*

# Inhibition of multiple sclerosis–associated retrovirus as biomarker of interferon therapy

Giuseppe Mameli,[1] Caterina Serra,[1] Vito Astone,[1] Massimiliano Castellazzi,[2] Luciana Poddighe,[1] Enrico Fainardi,[2] Walter Neri,[3] Enrico Granieri,[2] and Antonina Dolei[1]

[1]*Section of Microbiology, Department of Biomedical Sciences and Center of Excellence for Biotechnology Development and Biodiversity Research, University of Sassari, Sassari, Italy*
[2]*Multiple Sclerosis Center, Department of Neurology, University of Ferrara, Ferrara, Italy*
[3]*Neurology Operative Unit, Presidio Ospedaliero AUSL, Forlì, Italy*

**The authors performed a longitudinal evaluation of multiple sclerosis (MS) patients, during 1 year of therapy with interferon-$\beta$ (IFN-$\beta$), by clinical examination and detection of presence in the blood and viral load of MS-associated retrovirus (MSRV), by MSRV*env*-specific, fully quantitative, real time reverse transcriptase–polymerase chain reaction (RT-PCR). MSRV load in the blood was directly related to MS duration and fell below detection limits within 3 months of IFN therapy; one patient had strong progression, accompanied by total MSRV rescue. These findings suggest that evaluation of plasmatic MSRV could be considered the first prognostic marker for the individual patient, to monitor disease progression and therapy outcome.** *Journal of NeuroVirology* (2008) **14,** 73–77.

**Keywords:** biomarker; interferon therapy; MSRV/HERV-W endogenous retrovirus; multiple sclerosis

## Introduction

Multiple sclerosis (MS) is a complex disease of the central nervous system, in which several pathophysiological mechanisms are involved (inflammation, demyelination, axonal damage, and repairing). Over the years, progressive disability and irreversible deficit lead the majority of patients to chronic neurological deficit. MS has a variability of pathological features, clinical symptoms, and disease courses (Berger and Reindl, 2007). The etiology is still unknown, and the pathogenesis is likely to be autoimmune. Infectious agents have been suggested either as etiologic factors, cofactors, or triggering events (Granieri, 1997; Ascherio and Munger, 2007). The incidence of MS in Italy ranges from 4 to 6 new cases per 100,000 people and an increasing temporal trend is detected in some areas (Granieri *et al*, 2007; Pugliatti *et al*, 2005). A demanding task is to predict to the individual patient the clinical course, the timing of relapses, the rate of disability progression, as well as the outcome of ongoing treatments: in very truth, no biological marker for disease prognosis and progression of disease and therapy monitoring has been established in MS yet (Fainardi *et al*, 2004; Berger and Reindl, 2007; Singh *et al*, 2007; Fossey *et al*, 2007). Currently, disease progression is monitored based on the relapse rate, neurological deterioration, and evidence of disease activity on brain magnetic resonance imaging (MRI) scans; however, radiological manifestations are only weakly correlative with the patient's clinical course (Singh *et al*, 2007).

Human endogenous retroviruses (HERVs) of the W family have been proposed as environmental cofactors triggering MS immunopathogenic phenomena in a predisposing genetic background (Perron *et al*, 1997; Dolei *et al*, 2002; Antony *et al*, 2004). We studied MSRV (MS-associated retrovirus), the founder member of the HERV-W family, in MS patients in various temporal and clinical stages of the

Address correpondence to Antonina Dolei, Section of Microbiology, Department of Biomedical Sciences, viale San Pietro 43B, I-07100, Sassari, Italy. E-mail: doleivir@uniss.it

This work was supported partly by grants from Fondazione Italiana Sclerosi Multipla Onlus (grant no. 2003/R/21), Ministero Università e Ricerca PRIN 2005. G.M. was supported by a training research fellowship of Fondazione Italiana Sclerosi Multipla Onlus (no. 2005/B/2).

Received 11 June 2007; revised 10 August 2007; accepted 20 September 2007.

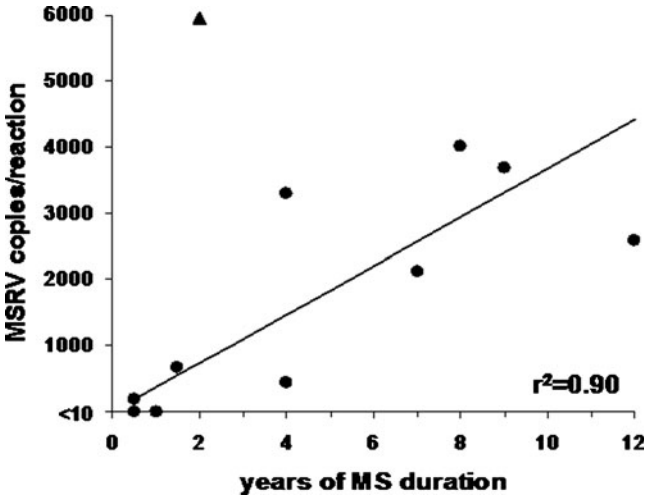


**Figure 1** Correlation of plasmatic viral load to the years of MS duration at study entry. Each point represents an individual; data of patient F02 are reported as (▲). $r^2$ is the correlation coefficient. MSRV load is expressed as copy number/reaction of real time RT-PCR. See text for details.

disease (Dolei *et al*, 2002), in follow-up evaluations (Sotgiu *et al*, 2002, 2006a), as well as in the conversion to full-blown MS of patients suffering of optical neuritis (Sotgiu *et al*, 2006b). In all cases, the data showed a striking parallelism between MS behavior and MSRV presence and/or load in patient's blood and spinal fluid. We found also that MSRV/HERV-W is highly expressed in MS brains, and that its env protein accumulates particularly in the core of active lesions, in cells resembling astrocytes (Mameli *et al*, 2007a). Cultured peripheral blood mononuclear cells from MS patients and from MSRV(+) healthy individuals release free virus in culture fluids (Serra *et al*, 2003), and MSRV production is stimulated by cell treatment with cytokines shown to be detrimental in MS patients, such as tumor necrosis factor-$\alpha$ (TNF-$\alpha$), interleukin-6 (IL-6), and interferon-$\gamma$ (IFN-$\gamma$), whereas IFN-$\beta$, which is used in MS therapy, causes a dramatic reduction of MSRV yields. Based on these premises, we performed a longitudinal study on a group of MS patients undergoing 1 year of therapy with IFN-$\beta$.

## Results

*MSRV load*

At study entry, mean viral load in the plasma was $2089.2 \pm 1999.0$ copies/reaction, and individual values were directly related to the years of MS duration (Figure 1; correlation coefficient = .9, despite the small number of patients under study).

The effect of IFN treatment on MSRV load is reported on Figure 2. For two patients we obtained also samples at 2 and 30 days after study entry, and we found that complete MSRV inhibition could occur as early as 48 h after the first IFN shot (Figure 2A). As shown in Figure 2B, after 3 months of therapy, MRSV was below detections limits in all patients (mean copies $2.3 \pm 4.8$, range 0 to 13, $P < .0000001$).

The inhibition of MSRV circulation remained stable throughout the entire year of therapy in all patients but one. At 12 months mean MSRV for all 11 patients was $440.1 \pm 1456.0$ (range 0 to 4830, $P < .0000001$); mean MSRV copies of the 10 complete responders: $1.1 \pm 3.5$ (range 0 to 11, $P < .0000001$). The nonresponder individual (Patient F02, a 39-years-old male, whose data are reported in Figure 1 by a ▲) was discordant from the other patients since study entry: Despite an history of only 2 years of MS duration, he had the highest viral load (Figure 1), and he restarted MSRV production after 6 months of therapy; at 12 months he had MSRV values similar to the pretherapy ones (Figure 2B).

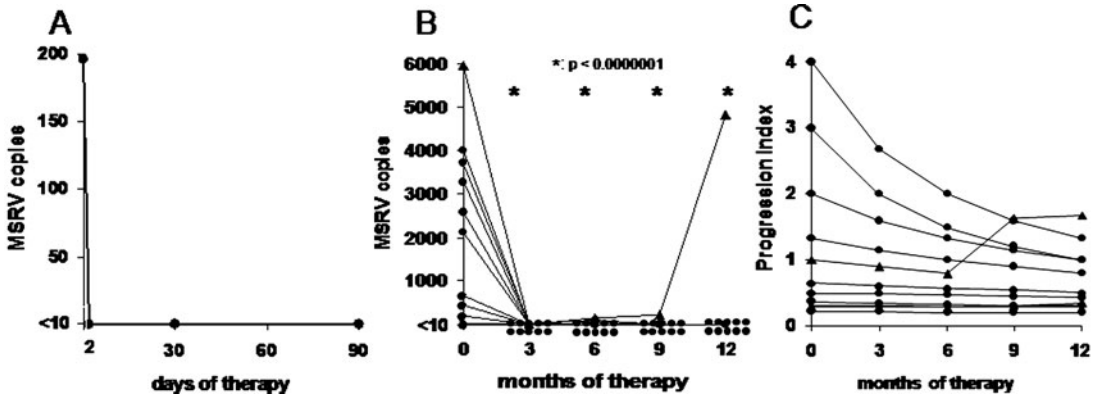


**Figure 2** Plasmatic viral load and disease outcome during 12 months of therapy with IFN$\beta$. Each point represents an individual; data of patient F02 are reported as (▲). (**A**) Rapid MSRV response to IFN, as detected in one MS patient, analyzed at 2, 30, and 90 days of therapy. (**B**) MSRV copy numbers during time. Asterisks indicate the *P* value obtained from variance analysis of the mean copy numbers during IFN treatment with respect to that of study entry. (**C**) disability status of each patient, expressed as Progression Index (PI: EDSS status divided by the years of MS duration). Variance analysis of mean EDSS values showed not statistically significant differences between study entry and after IFN therapy (not shown).

*Clinical evaluation*
On admission to the study, mean Expanded Disability Status Scale (EDSS) was $2.3 \pm 0.7$ (range 1.5 to 3.5), and mean exacerbations/year in the previous 2 years were $1.36 \pm 0.7$ (range 1 to 3). After 12 months of therapy, mean EDSS was $2.6 \pm 1.2$ (range 1.5 to 5, difference not significant at variance analysis) and mean exacerbations/year were $0.18 \pm 0.4$ ($P = .0014$). For each patient the progression index (PI: the EDSS status divided by the years of MS duration) throughout the 12 months of therapy was calculated. As shown in Figure 2C, during IFN therapy the PI was reduced for the majority of the patients, being the effect of IFN more pronounced in the persons with $PI > 1$ (that were patients with disease duration of less than 2 years, not shown). Of the two progressors, one was patient F02, the one discordant from the other patients with respect to MSRV load. Parallel to MSRV rescue, from month 6 onward his disease progressed, and within the last 6 months of therapy, his EDSS dramatically changed (EDSS from 2 to 5, PI from 1 to 1.67). The other patient had a milder progression, barely detectable in Figure 2C (PI from 0.29 to 0.36), without MSRV rescue: she was a 39-year-old female, with the oldest MS duration (12 years) and had EDSS = 3.5 at study entry.

## Discussion

We are aware of the limits of our study, namely a small number of patients and 12 months of observation only. However, the study was performed in a longitudinal fashion, and it is the first one that follows up the expression of an HERV *in vivo,* under therapy, and at different time intervals. Beside short-term clinical efficacy (14-fold reduction of mean exacerbation/year), IFN therapy caused a prompt and complete inhibition of MSRV circulation. Only one patient showed an early and strong disease progression, which was paralleled by complete restoration of MSRV production.

The role of MSRV in MS, as cofactor or epiphenomenon, cannot be defined so far. As for the former possibility, the env proteins of retroviruses have potential immunopathogenic properties, causing neuroinflammation, neurodegeneration, and endoplasmic reticulum stress responses (Antony *et al*, 2007). MSRV produces extracellular virions with gliotoxic (apoptotic), fusogenic and superantigenic properties, and causes a T cell–mediated neuropathology *in vivo* in humanized, severely compromised immunodeficient (SCID) mice (Firouzi *et al*, 2003). Moreover, a complex virus interplay might occur, as MSRV expression is transactivated by herpesviruses (Ruprecht *et al*, 2006) and the simultaneous presence of HERV and herpesvirus antigens has synergistic effects on cell-mediated immune responses (Brudek *et al*, 2004). Activities strikingly concordant with MSRV findings were reported for syncytin-1, an env protein encoded by a replication incompetent element belonging to the same HERV-W family, as MSRV, and located on chromosome 7q21–22, in a region of candidate genetic susceptibility for MS. Syncytin-1 is involved in embryo implantation during pregnancy (Mi *et al*, 2000), but is expressed also in the brains of MS patients (Antony *et al*, 2004); in astrocytes it modulates an inflammatory cascade, leading to adverse effects on oligodendrocyte proteins involved in myelin formation (Antony *et al*, 2007). As previously shown for MSRV (Serra *et al*, 2003), MS-detrimental cytokines activate syncytin-1 promoter, whereas the MS-protective IFN-$\beta$ is inhibitory (Mameli *et al*, 2007b). Even if it is a mere epiphenomenon, however, MSRV detection in MS patients can be useful: MSRV presence in cerebrospinal fluid (CSF) of monosymptomatic optic neuritis patients was associated with increased conversion to definite MS (Sotgiu *et al*, 2006b). Accordingly, by follow up of otherwise identical MS patients, patients who had had MSRV(+) spinal fluids showed worse progression (Sotgiu *et al*, 2002; Sotgiu *et al*, 2006a), suggesting MSRV as a negative prognostic marker.

The present data indicate that (i) MSRV load in the blood is directly related to MS duration; (ii) IFN therapy reduces the virus rapidly below detection limits; (iii) strong progression with therapy failure is accompanied by MSRV rescue. These findings are in favor of the possibility that detection of plasmatic MSRV can be considered the first prognostic marker for the individual patient, which can be useful to monitor disease progression and therapy outcome.

## Materials and methods

*Patients*
Eleven patients with definite relapsing-remitting (RR) MS according to current criteria (McDonald *et al*, 2001; Lublin and Reingold, 1996; Poser *et al*, 1983), and followed as outpatients in the Day Hospital of the Multiple Sclerosis Center, University of Ferrara, were consecutively enrolled: 6 females and 5 males, mean age $34.0 \pm 8.6$ years (range 21 to 46 years), MS duration $4.5 \pm 3.9$ years (range 0.5 to 12 years). At study entry, all patients were clinically stable because they had had at least two exacerbations in the previous 2 years, were relapse-free in the preceding 3 months, had $\leq$3.5 points on the Expanded Disability Status Scale (EDSS) score (Kurtzke, 1983), and had not had a significant progression in disability, as assessed by an increase of 1 EDSS point in the last 2 years. Mean number of relapses in the 2 years before start of treatment was $2.36 \pm 0.50$ (range 2 to 3).

No patients had received any potential disease-modifying therapies during the 6 months before inclusion in the study. The study was approved by the Regional Committee for Medical Ethics in

Research and all patients gave informed consent. Types of IFN and regimens were: IFN-$\beta$1b (Betaferon; Shering, Berlin; 8 MIU subcutaneously, every other day, $N=4$); IFN-$\beta$1a (Avonex; Biogen, San Diego; 6 MIU, weekly intramuscular injection, $N=3$); and INF-$\beta$1a Serono, Geneva; 6 MIU ($N=3$) and 12 MIU ($N=1$) subcutaneously, three times/week, $N=4$. None of the patients discontinued IFN-$\beta$ therapy for ineffectiveness or other reasons over the study period.

Routine clinical examination and blood withdrawal were blindly performed at study entry and every 3 months. Additional interventions were performed if required for problems such as relapses, tolerability of therapies, etc. Follow-up neurological evaluation included documenting ongoing relapses, testing current EDSS, and determining side effects or adverse events. In this setting, a total of two clinical attacks were recorded during treatment (one between months 3 and 6, and one between months 6 and 9), whereas no significant side effects or adverse events were observed during therapy. The two patients who underwent exacerbations were treated with intravenous methylprednisolone, 1000 mg/day for 5 consecutive days. Serum samples were drawn under sterile conditions, coded, frozen, and stored in aliquots at $-70^{\circ}$C until the assay. To exclude short-term IFN-$\beta$ effects, all samples were taken at least 12 to 36 h after IFN-$\beta$ injection. None of the patients had been receiving corticosteroids when these samples were collected. All analyses were carried out under exactly the same conditions.

*MSRV evaluation*

Plasma samples were treated as described (Dolei *et al*, 2002; Mameli *et al*, 2007a), transported frozen, and tested concurrently under strict blind code. Extracellular polyA(+) virionic RNAs were extracted from 1 ml of cell-free plasma by mRNA Dynabeads (Dynal Biotech, Oslo, Norway). Evaluation of MSRV copies was obtained by retrotranscription and fully quantitative real-time polymerase chain reaction (PCR) amplification, as described (Dolei *et al*, 2002; Mameli *et al*, 2007a).The assay employed primers specific for MSRV/HERV-W-*env* and external calibration curves of a plasmidic construct containing an *env* gene fragment (Mameli *et al*, 2007a). Data were expressed as copy numbers/PCR reaction mixture. We considered 10 copies/reaction as the detection limits of real-time PCR assay, in line with published reports (Contreras-Galindo *et al*, 2006; Tuke *et al*, 2004). To ensure correct amplification and retrotranscription (Mameli *et al*, 2007a), controls included PCR of RNAs not exposed to reverse transcriptase (RT) with primers specific for the $\beta$-globin gene (primer pair PC04/GH20; Synthetic Genetics) or with MSRV/HERV-W–specific primers (to ensure the absence of contaminating cellular DNA sequences and of endogenous retroviral DNA sequences, respectively), and PCR of cDNA samples without template (negative control) and samples of human cellular DNA (positive control). Cellular RNA from peripheral blood mononuclear cells (PBMCs) of individuals shown previously to be negative for circulating MSRV (and whose PBMCs did not release or transcribe MSRV/HERV-W in culture; Serra *et al*, 2003) was also included. Presence/absence of MSRV/HERV-W was confirmed in repeated assays of the same sample. The specificity of the amplified products was confirmed by dideoxy sequencing (Mameli *et al*, 2007a).

*Statistics*

The significance of the results was evaluated by means of the Epi InfoDatabase and Statistics Software Program, Version 3.3.2 (CDC/WHO, Atlanta, GA).

## References

Antony JM, Ellestad KK, Hammond R, Imaizumi K, Mallet F, Warren KG, Power C (2007). The human endogenous retrovirus envelope glycoprotein, syncytin-1, regulates neuroinflammation and its receptor expression in multiple sclerosis: a role for endoplasmic reticulum chaperones in astrocytes. *J Immunol* **179:** 1210–1224.

Antony JM, Van Marle G, Opii W, Butterfield DA, Mallet F, Yong VW, Wallace JL, Deacon RM, Warren K, Power C (2004). Human endogenous retrovirus glycoprotein-mediated induction of redox reactants causes oligodendrocyte death and demyelination. *Nat Neurosci* **7:** 1088–1095.

Ascherio A, Munger KL (2007). Environmental risk factors for multiple sclerosis. Part I: The role of infection. *Ann Neurol* **61:** 288–299.

Berger T, Reindl M (2007). Multiple sclerosis: disease biomarkers as indicated by pathophysiology. *J Neurol Sci* **15:259:** 21–26.

Brudek T, Christensen T, Hansen HJ, Bobecka J, Moller-Larsen A (2004). Simultaneous presence of endogenous retrovirus and herpes virus antigens has profound effect on cell-mediated immune responses: implications for multiple sclerosis. *AIDS Res Hum Retroviruses* **20:** 415–423.

Contreras-Galindo R, Gonzalez M, Almodovar-Camacho S, Gonzalez-Ramirez S, Lorenzo E, Yamamura Y (2006). A new Real-Time-RT-PCR for quantitation of human endogenous retroviruses type K (HERV-K) RNA load in plasma samples: increased HERV-K RNA titers in HIV-1 patients with HAART non-suppressive regimens. *J Virol Methods* **136:** 51–57.

Dolei A, Serra C, Mameli G, Pugliatti M, Sechi G, Cirotto MC, Rosati G, Sotgiu S (2002). Multiple sclerosis-associated retrovirus (MSRV) in Sardinian MS patients. *Neurology* **58:** 471–473.

Fainardi E, Rizzo R, Melchiorri L, Castellazzi M, Govoni V, Caniatti L, Paolino E, Tola MR, Granieri E, Baricordi OR

(2004). Beneficial effect of interferon-beta 1b treatment in patients with relapsing-remitting multiple sclerosis is associated with an increase in serum levels of soluble HLA-I molecules during the first 3 months of therapy. *J Neuroimmunol* **148:** 206–211.

Firouzi R, Rolland A, Michel M, Jouvin-Marche E, Hauw JJ, Malcus-Vocanson C, Lazarini F, Gebuhrer L, Seigneurin JM, Touraine JL, Sanhadji K, Marche PN, Perron H (2003). Multiple sclerosis-associated retrovirus particles cause T lymphocyte-dependent death with brain hemorrhage in humanized SCID mice model. *J NeuroVirol* **9:** 79–93.

Fossey SC, Vnencak-Jones CL, Olsen NJ, Olsen NJ, Sriram S, Garrison G, Deng X, Crooke PS, Aune TM (2007). Identification of molecular biomarkers for multiple sclerosis. *J Mol Diagn* **9:** 197–204.

Granieri E (1997). Editorial. Analytic approaches to the study of etiological factors in multiple sclerosis. Guidelines for future epidemiologic studies. Introduction. *Neurology* **49(Suppl. 2):** S2–S3.

Granieri E, Oikonomou NT, De Gennaro R, Govoni V, Tola MR, Caniatti L, Fainardi E, Casetta I (2007). Multiple sclerosis in the province of Ferrara, Italy. Evidence for an increasing trend. *J Neurol* 254:1642–1648.

Kurtzke JF (1983). Rating neurological impairment in multiple sclerosis: an expanded disability scale (EDSS). *Neurology*. **33:** 1444–1452.

Lublin DF, Reingold SC (1996). Defining the clinical course of multiple sclerosis: results of an international survey. *Neurology* **46:** 907–911.

Mameli G, Astone V, Arru G, Marconi S, Lovato L, Serra C, Sotgiu S, Bonetti B, Dolei A (2007a). Brains and peripheral blood mononuclear cells of multiple sclerosis (MS) patients hyperexpress MS-associated retrovirus/HERV-W endogenous retrovirus, but not Human herpesvirus 6. *J Gen Virol* **88:** 264–274.

Mameli G, Astone V, Khalili K, Serra C, Sawaya BE, Dolei A (2007b). Regulation of the syncytin-1 promoter in human astrocytes by multiple sclerosis-related cytokines. *Virology* **362:** 120–130.

McDonald WI, Compston A, Edan G, Goodkin D, Hartung HP, Lublin FD, McFarland HF, Paty DW, Polman CH, Reingold SC, Sandberg-Wollheim M, Sibley W, Thompson A, van den Noort S, Weinshenker BY, Wolinsky JS (2001). Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the Diagnosis of Multiple Sclerosis. *Ann Neurol* **50:** 121–127.

Mi S, Lee X, Li X, Veldman GM, Finnerty H, Racie L, LaVallie E, Tang XY, Edouard P, Howes S, Keith Jr JC, McCoy JM (2000). Syncytin is a captive retroviral envelope protein involved in human placental morphogenesis. *Nature* **403:** 785–789.

Perron H, Garson JA, Bedin F, Beseme F, Paranhos-Baccala G, Komurian-Pradel F, Mallet F, Tuke PW, Voisset C, Blond JL, Lalande B, Seigneurin JM, Mandrand B (1997). Molecular identification of a novel retrovirus repeatedly isolated from patients with multiple sclerosis. The Collaborative Research Group on Multiple Sclerosis. *Proc Natl Acad Sci U S A* **94:** 7583–7588.

Poser CM, Paty DW, Scheimberger L, McDonald WI, Davis FA, Ebers GC, Johnson KP, Sibley WA, Silberberg DH, Tourtellotte WW (1983). New diagnostic criteria for MS: guidelines for research protocols. *Ann Neurol* **13:** 227–231.

Pugliatti M, Riise T, Sotgiu MA, Sotgiu S, Satta WM, Mannu L, Sanna G, Rosati G (2005). Increasing incidence of multiple sclerosis in the province of Sassari, northern Sardinia. *Neuroepidemiology* **25:** 129–134.

Ruprecht K, Obojes K, Wengel V, Gronen F, Kim KS, Perron H, Schneider-Schaulies J, Rieckmann P. (2006). Regulation of human endogenous retrovirusW protein expression by herpes simplex virus type 1: implications for multiple sclerosis. *J NeuroVirol* **12:** 65–71.

Serra C, Mameli G, Arru G, Sotgiu S, Rosati G, Dolei A (2003). In vitro modulation of the multiple sclerosis (MS)-associated retrovirus (MSRV) by cytokines: implications for MS pathogenesis. *J NeuroVirol* **9:** 637–643.

Singh MK, Scott TF, Laframboise WA, Hu FZ, Post JC, Ehrlich GD (2007). Gene expression changes in peripheral blood mononuclear cells from multiple sclerosis patients undergoing beta-interferon therapy. *J Neurol Sci* **258:** 52–59.

Sotgiu S, Arru G, Mameli G, Serra C, Pugliatti M, Rosati G, Dolei A (2006a). MSRV in early multiple sclerosis: a six-year follow-up of a Sardinian cohort. *Mult Scler* **12:** 698–703.

Sotgiu S, Arru G, Söderström M, Mameli G, Serra C, Dolei A. (2006b). MSRV and optic neuritis. *Mult Scler* **12:** 357–359.

Sotgiu S, Serra C, Mameli G, Pugliatti M, Rosati G, Arru G, Dolei A (2002). Multiple sclerosis (MS)-associated retrovirus and MS prognosis: an observational study. *Neurology* **59:** 1071–1073.

Tuke PW, Hawke S, Griffiths PD, Clark DA (2004). Distribution and quantification of human herpesvirus 6 in multiple sclerosis and control brains. *Mult Scler* **10:** 355–359.