Azathioprine in Multiple Sclerosis

Paolo Invernizzi*, Maria Donata Benedetti, Sarah Poli and Salvatore Monaco*

Department of Neurological and Visual Sciences, Section of Clinical Neurology, University of Verona, Verona, Italy

Abstract: Azathioprine is an immunosuppressive and steroid-sparing purine analogue, used in the treatment of several autoimmune diseases. In multiple sclerosis, available evidence suggests that oral azathioprine reduces relapse rates, provides a slight benefit on disability, and reduces new inflammatory lesions. Here, we focus on molecular mechanisms of Azathioprine and on its usefulness in multiple sclerosis.

Key Words: Azathioprine, multiple sclerosis, immunosuppression, thiopurine methyltransferase, tolerability, relapses, disability, immunomodulators.

INTRODUCTION

Multiple sclerosis (MS) is an acquired chronic, relapsing and remitting autoimmune disorder of the central nervous system (CNS) [1]. The pathological hallmark of MS is the plaque, a focal inflammatory lesion of the white matter produced by a number of sequential events, including homing of activated T cells, matrix metalloproteinase-induced damage to endothelial basal lamina, loss of the blood-brain-barrier (BBB) integrity, perivascular cellular infiltration, demyelination, edema, and axonal degeneration [2]. Depending on their location, white matter plaques may cause focal sensory, pyramidal, visual, cerebellar or brainstem signs. Occasionally, lesions occur in the cortical gray matter, in which case they are pathologically characterized by myelin/axonal injury and microglial activation, in the absence of cellular infiltration and BBB breakdown [3]. Patients with cortical lesions may present with neuropsychiatric and focal cortical symptoms encompassing aphasia, alexia, alien hand, tremor and seizures [4].

In terms of genetics, epidemiology, and clinical course, MS is a heterogeneous disorder, since B and T cell responses are likely triggered in a different number of ways [5]. Therefore, treatment strategies need to broadly target common pathogenic pathways, in addition to selecting optimized drug regimens for given subgroups of MS patients. Effective therapies in MS should prevent disease relapses and progression by balancing the immune network, rather than inducing immune suppression, as most of current treatments do. While a large number of therapeutic strategies are currently available in MS, it remains unknown whether some of the drugs effectively used in earlier treatment regimens have fallen short of clinical efficacy because of intrinsic inadequacy or due to inadequate assessment in appropriate trials. Here we review old and recent literature on azathioprine efficacy in MS.

*Address correspondence to these author at the Department of Neurological and Visual Sciences, Section of Clinical Neurology, University of Verona, Piazzale L.A. Scuro, 10, 37134 Verona, Italy; Tel: (39) 045-8124285; Fax: (39) 045-8124873;
E-mail: p_invernizzi@yahoo.it, salvatore.monaco@univr.it

AZATHIOPRINE: PHARMACOKINETICS, PHARMACOGENETICS AND SIDE EFFECTS

Azathioprine (Aza), the 1-methyl-4-nitro-5-imidazolyl derivative of 6-mercaptopurine (Fig. **1**), is a prodrug which acts as an antimetabolite, due to its structural analogy with purines.

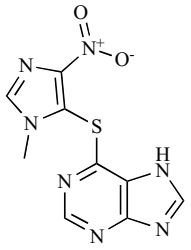

Fig. (1). Structure of azathioprine.

After administration, Aza is well absorbed through the gastrointestinal tract, and is converted into 6-mercaptopurine (6-MP) in the liver; 6-MP is converted in the liver and gut by phase I (oxidation) and phase II (conjugation) reactions: (i) hypoxanthine guanine phosphoribosyltransferase (HGPRT), generating 6-thioguanine –nucleotides (6-TGN), which are responsible for the immunosuppressive and toxic activity of Aza; (ii) thiopurine methyltransferase (TPMT) leading to methylation of 6-MP and formation of 6-methylmercaptopurine (6-MMP); (iii) xanthine oxidase, which leads to the formation of 6-thiouric acid (Fig. **2**). Since the latter two pathways lead to the production of largely inactive compounds, a reduced TPMT or xanthine oxidase activity (i. e., *via* inherited deficiency or allopurinol administration, respectively) induces an increased production of 6-TGN and the potential development of myelotoxicity. The half-life of Aza and its metabolic analogs 6-MP is about 2 hours, although a significant inter- and intra-patient variation can be observed.

In humans, TPMT activity is widely expressed in many tissues and red cells and is inherited as an autosomal codominant trait. In Caucasians, a trimodal distribution is seen, with 0.3-0.6% individuals having low (L) or undetectable activity, 10% having intermediate activity, and the remaining 90% having high (H) activity [6]. The molecular

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basis for this variability, formerly designated as $TPMT^{L}$/$TPMT^{L}$, $TPMT^{L}$/$TPMT^{H}$, and $TPMT^{H}$/$TPMT^{H}$, is the allelic combination of the H-activity wild-type *TPMT*1* with L-activity polymorphic variants *TPMT*2*, **3A* (the most common in Caucasians), **3B* and **3C*. [7, 8]. Therefore, homozygosity for a variant allele (e.g. *TPMT *3A*/**3A*) results in low TPMT activity, as opposed to the intermediate activity seen in heterozygosity (e.g. *TPMT*1*/**3A*), and high activity in subjects homozygous for wild-type alleles (*TPMT *1*/**1*).

Although genotyping is reasonably accurate in predicting TPMT phenotype, most clinicians prefer to dose TPMT activity [9], as ample variations in its activity are seen in *TPMT *1*/**1* subjects. Assessment of TPMT activity, which inversely relates to erythrocyte 6-TGN concentration [10], provides the best indication of the patient's ability to metabolize thiopurines. Subjects with TPMT deficiency have very high concentration of 6-TGNs, thus being at risk of myelotoxicity, although approximately three-quarters of cases of bone marrow suppression occur in subjects without TPMT deficiency [11]. Therefore, testing for TPMT status should be implemented with blood count monitoring. Conversely, high TPMT activity and elevated 6-MMP levels have been shown to predispose to hepatotoxicity in selected conditions [12]. For individuals with very low TPMT activity, an alternative immunosuppressant should be considered, although frequent measurement of erythrocyte 6-TGNs concentrations and full blood counts can facilitate a safer use of Aza.

Additional side effects of Aza include occasional liver dysfunction, cholestatic jaundice, hepatic venoocclusive disease, and skin rash.

MECHANISMS OF ACTION

The thiopurine drugs 6-MP and Aza are purine antimetabolites with antileukemic and immunosuppressants properties. In earlier experimental and clinical studies, Aza proved to be much less toxic than 6-MP and produced a better prolongation of allograft survival. For this reason, Aza was introduced in clinical practice and remained the mainstay of immunosuppression for almost three decades. The immunosuppressive activity of Aza and 6-MP is due to their interference with nucleic acid synthesis during the cellular multiplication that follows B and T cell activation. Aza and its metabolic products act as purine antagonists and inhibit the synthesis of RNA and DNA when incorporated into replicating nucleic acid, therefore stopping nucleic acid assembly [13]. In addition, they also block the *de novo* pathway of purine synthesis by formation of thio-inosinic acid. The latter effect is readily evident on lymphocytes, which lack a salvage pathway for purine synthesis. In addition to their lymphotoxicity, these purine analogs inhibit the T-cell-dependent antibody-mediated response, by interfering with CD28 costimulation of alloreactive T lymphocytes, a process mediated by GTPase Rac1 [14]. The latter effect is due to conversion of 6-thioguanine, a metabolic product of Aza, into 6-thioguanine triphosphate (6-thioGTP), a compound which competes with GTP and binds to the GTPase Rac1. The interaction between 6-thioGTP and GTPase Rac1 ultimately leads to blockade of Rac1 and conversion of the normal costimulatory CD28 signal into an apoptotic signal, with ensuing death of activated lymphocytes. In resting B cells, purine analogs do not greatly interfere with plasma cell mRNA synthesis and antibody production.

Aza has still many clinical uses in internal medicine, rheumatology and neurology [15], since, in addition to its immunosuppressant activity, it helps in reducing the dose of other drugs, such as steroids. In particular, adding steroids to Aza is beneficial in reducing the progression of myasthenia gravis and in achieving long-term control of other neurologic conditions, such as multiple sclerosis, Lambert-Eaton myasthenic syndrome and chronic inflammatory demyelinating polyradiculoneuropathy [16]. It should, however, be noted that several lines of evidence suggest that to be effective Aza requires at least six months of treatment, or longer.

An increasing number of immunosuppressive agents are now available, each acting at different steps of the immunological response (Fig. **2**). These agents include (i) glucocorticoids, which regulate gene expression; (ii) cyclophosphamide, an alkylating agent that is useful in diseases with pathogenic autoantibodies; (iii) kinase and phosphatase inhibitors (cyclosporin A, tacrolimus, rapamycin, type IV PDE inhibitors, p38 kinase inhibitors), which regulate signalling decoding [17].

In addition to inhibitors of *de novo* purine synthesis (azathioprine, mycophenolate, mofetil, and mizoribine), antimetabolites include inhibitors of *de novo* pyrimidine synthesis (leflunomide and brequinar). Methotrexate, due to its action on dihydrofolate reductase (antifolate), inhibits *de novo* pyrimidine and purine synthesis.

EFFICACY OF AZA IN MULTIPLE SCLEROSIS

Although the aetiology of MS is still largely unknown, a widely accepted conceptual framework postulates that autoimmune mechanisms, in conjunction with a genetic predisposing background, play a central role in its pathogenesis [18]. While considerable therapeutic challenges remain for the treatment of MS, the major goal of management is to avoid relapses and to stop disease progression. Among disease-modifying treatments for MS, Aza represents one of the oldest drugs that is still in use today.

Published data about the efficacy of Aza derive from a number of randomized clinical trials (RCTs), most of which involving small numbers of patients. These trials had been conducted in the "pre-interferon era", i.e. before 1993, when the first large RCT on interferon beta (INFB) in relapsing-remitting (RR) MS was published [19]. Moreover, a standardized classification of the different clinical forms of MS was obtained by consensus only in 1996 [20], and, therefore, inclusion criteria previously used were not uniform among studies. Finally, most RCTs on Aza were conducted before magnetic resonance imaging (MRI) was available in many MS centres.

In 1991, Yudkin *et al*. [21] published a meta-analysis of the seven existing Aza RCTs [22-28]. Most of these studies included less than one hundred patients per group, except for the British and Dutch MS Aza Trial Group study [24], involving 174 patients in the Aza group and 180 patients in the placebo group. The total number of patients included in Yudkin's meta-analysis was 392 for Aza and 401 for pla-

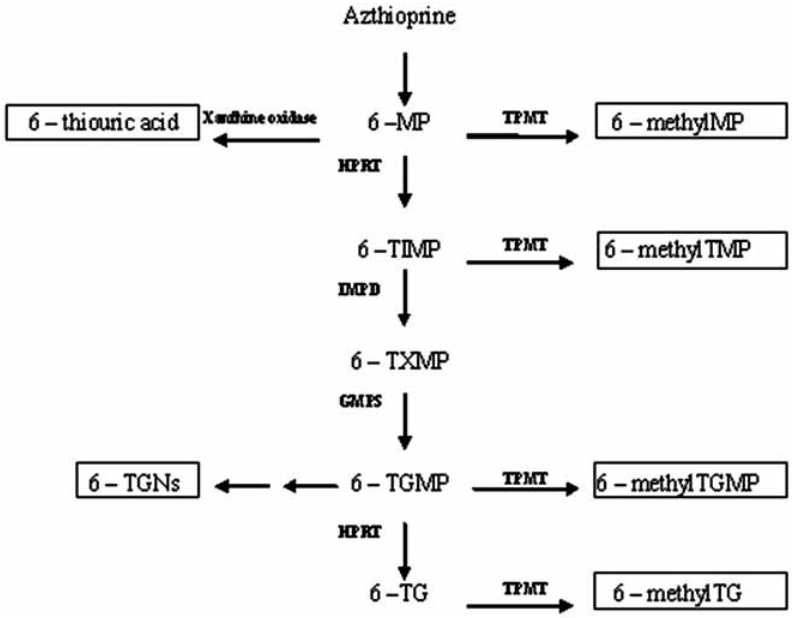

Fig. (2). Metabolism of azathioprine and 6-mercaptopurine. GMPS: guanosine monophosphate synthetase; HPRT: hypoxanthine phosphoribosyl-transferase; IMPD: inosine monophosphate dehydrogenase; TG: thioguanine; TGMP: thioguanine monophosphate; TGN: thioguanine nucleotides; TIMP: thioinosine monophosphate; TXMP: thioxanthosine monophosphate.

cebo. However, follow-up data were available only for 253 Aza and 277 placebo at 2 years, and for 200 Aza and 215 placebo at 3 years. In three RCTs only patients with RR course were enrolled [22,23,28], but in the remaining ones progressive forms were also included [24-27]. Trials in which Aza was given in association with other drugs (e.g., prednisone) were excluded from meta-analysis. The daily Aza dose ranged from 2.2 to 3.0 mg/kg, and in some studies [27,28], the dose was adjusted to maintain leukocyte count between 3,000-4,000/μl. Two end-points were identified: (i) the effect on disability, measured as the mean change in Kurtzke Expanded Disability Status Score (EDSS) [29], as compared to baseline, and (ii) the probability to be free from relapse after 1, 2 and 3 years of treatment. In all RCTs, mean EDSS increased both in treated and untreated groups. However, after 1 year of treatment, a small, even if not statistically significant, benefit in Aza group was observed, and this trend was confirmed at 2 years (-0.22), and at 3 years (-0.24). Moreover, the efficacy of Aza in reducing relapse risk was evident in this meta-analysis. Accordingly, the odds ratio to be free from relapse for treated cases *vs*. controls was 1.51 at 1 year, 2.04 at 2 years, and 1.97 at 3 years ($p< 0.01$). Intriguingly, the effect of Aza either on disability and relapse risk seems to increase in time.

With the approval of IFNB and glatiramer acetate (GA) for the treatment of RR MS, RCTs involving Aza were interrupted and, as a result, no RCTs analysed its effect on MRI parameters. Only one retrospective study examined Aza impact on MRI in MS, showing that after a mean time of 2.5 years patients treated with Aza presented a mean reduction of 28.4% of T2 lesion load compared to controls, in whom, conversely, a mean increase of 19.3% was observed [30]. A prospective study of Aza effect in reducing new MRI brain lesions was recently published by Massacesi *et al.* (2005) [31]. In 14 MS patients with RR course and MRI signs of inflammatory activity, the number of gadolinium-enhancing lesions and new T2-weighted hyperintense lesions was compared before and after the onset of Aza therapy in 6 monthly brain MRI scans. The median gadolinium-enhancing lesion number *per* MRI was 2 during the baseline period and 0 during treatment, with a mean reduction of 64% ($p < 0.001$). Also gadolinium-enhancing lesion volume showed a similar reduction (66%, $p < 0.001$), and the cumulative new T2 lesion number and volume *per* patient decreased consistently ($p < 0.02$ and $p < 0.05$ respectively). An ongoing Italian multicenter RCT will compare Aza and interferon-beta (IFNB) effects on clinical and neuroradiological outcomes in a large sample of RR MS patients.

COMPARISON OF IFNB, GA, AND AZA EFFICACY IN MS

IFNB is currently the most used and recognized treatment in RRMS. It has been approved more than ten years ago, after the publication of the first RCT suggesting its efficacy on disease activity. INFB can be administred at different doses and by different routes (subcutaneously or intramuscularly). The biological mechanisms of IFNB in MS have not been completely understood, but they probably involve activation and proliferation of suppressor T-cells, in addition to inhibition of T-cells ability to cross the blood-brain barrier and enter the CNS. In RCTs, the most evident clinical effect was the reduction of clinical relapse frequency in RR patients (from 18 to 32% against placebo at 2 years [19,32,33]). MRI analysis showed an even stronger effect in reducing new lesions number, a pivotal finding in assessing its clinical efficacy [34]. Conversely, the results on disability have been scanty [19,35].

In 2001, a Cochrane meta-analysis of data from existing RCTs of IFNB efficacy in RR MS was published [35]. Seven different RCTs [19,32,36,37,39,40], from 1993 to 1999, con-

tributed to this study, including 614 patients treated with INFB and 601 randomized to placebo. Although INFB studies included only RR patients, results of this meta-analysis are tentatively compared with those obtained in MS patients treated with Aza.

The most evident effect of the two therapies is that of reducing relapse rate. The relative risk *vs*. placebo of one or more exacerbation is as follows: (i) at 1 year follow-up, this risk is 0.73 for IFNB *vs*. 0.81 for Aza; (ii) at 2 year follow-up the risk is 0.79 for IFNB *vs*. 0.77 for Aza. The data about 3 year follow-up *vs*. placebo are lacking in IFNB RCTs, whereas in the Aza-treated group the relative risk is 0.83. Based on these data, it is safe to conclude that there is not evident difference between Aza and IFNB in preventing relapses. Moreover, it can be observed that the IFNB efficacy decreases after the first year of treatment, and it is not confirmed after two years of follow-up. On the other hand, while Aza is apparently slightly less effective than IFNB at 1 year, it maintains its efficacy through following years.

Regarding the progression of disability, it must be said that both drugs have a weak power. The mean change of EDSS *vs*. placebo at 2 years follow-up is -0.25 for IFNB and -0.22 for Aza. Therefore, based on this parameter, no clear difference exists between the two drugs. The tendency in reducing EDSS in Aza-treated patients *vs*. placebo is also evident at the 3 year follow-up, with a mean change of -0.24, whereas, at 3 year, data are lacking for IFNB.

Analysis of MRI in Aza-treated subjects are far less numerous than IFNB-treated ones, because studies involving Aza were mostly performed when MRI was not yet widely available.. The study published in 2005 by Massacesi *et al*. [31] showed an effect of Aza in suppressing new brain lesions similar to that observed with IFNB using the same study design [41-43].

Glatiramer acetate (GA) is a mixture of scrambled synthetic polypeptides containing L-alanine, L-glutamic acid, and L-tyrosine, which was found to be effective in suppressing experimental allergic encephalomyelitis [44]. Encouraging results obtained in a small clinical trial led to regulatory authorization to administer GA subcutaneously at daily doses of 20 mg [45]. In 2003, a Cochrane review of RCTs about the efficacy of GA therapy in RR MS patients was published [46]. Three RCTs were enrolled in the meta-analysis [45,47, 48], including 269 patients in the GA group, and 271 in the placebo group. The relative risk *vs*. placebo of one or more exacerbation reported by the meta-analysis was 0.77 at 1 year of follow-up, 0.87 at 2 years and 0.89, at 35 months. The mean change in EDSS was -0.33 at 2 years and -0.45 at 35 months. This data are not overtly different from those reported about IFNB or Aza.

A post-marketing review comparing the probability to be free from relapses at 2 years in MS patients treated with IFNB, GA, intravenous immunoglobulins, or Aza concluded for an equivalent efficacy of all these treatments[49]. However, the cost of therapy per year for IFNB and GA was 125 times higher than that of Aza. A more recent analysis of MS treatments used in UK concluded that IFNB, GA and Aza produce a similar reduction in the risk of relapses at 2 years (15-30%), and that a head to head comparison between these medications is worth to be tried [50]. A simulated cost-utility analysis in RR MS patients, based on trial-assessed drug efficacy, showed a slight preference for Aza; in the worst hypothetical scenario for Aza, the cost of each additional quality-adjusted life years (QALY) with IFNB would range between 413,000 and 1,308,000 euros in 2005 [51].

A pilot open study comparing Aza and IFNB efficacy and impact on quality of life was performed in a small sample of 32 RR MS patients [52]. They were allocated to one of three groups (11 IFNB, 10 Aza, 11 no treatment) according to the patient's choice. IFNB and Aza confirmed their comparable efficacy in reducing relapse rate *vs*. no treatment. However, the impact on quality of life was better for Aza, as a likely effect of its higher tolerability, or due to a more pronounced and persistent perception of the disease in patients treated with IFNB.

AZA AND IMMUNOMODULATORS: SIDE EFFECTS AND TOXICITY

In a population of 213 patients treated for a mean time of 4 years at the Lyon MS Hospital, no irreversible side effects of Aza were observed [53]. In 22 cases (10.3%) the treatment was stopped due to intolerance. Thirty-six patients (16.9%) presented transient side effects. In 29 cases (13.6%) reversible bone marrow hypoplasia was observed, with clinical expression only in 4 cases (1.9%). Gastrointestinal disturbances, including anorexia, abdominal pain, diarrhoea and vomiting were seen in 26 cases (12.2%), mostly at the onset of treatment, and in half cases suspension was necessary. Two patients (0.9%) presented eczema and 3 (1.4%) had alopecia. The incidence of infections was not increased in the treated sample, nevertheless 5 cases (2.3%) of herpes zoster and one case of HBV hepatitis were observed. The experience reported from the Florence MS Center was similar [54]. In 219 patients treated for a mean time of 4.16 years, Amato *et al*. observed 36 cases of leukopenia, 18 cases of increased hepatic enzymes, 17 with gastrointestinal complaints, and in a lower number of patients anemia, thrombocytopenia, and herpes zoster. Therapy was discontinued in 26 patients (11.9%), the most common cause of drop-out being gastroenteric disturbances.

Also IFNB is a generally well tolerated therapy, although patients often complain of disturbing side effects. In RCTs [35], 48% of IFNB-treated subjects presented flu-like symptoms, 28% fever, 26% myalgias or arthralgias, 17% fatigue, 50% headache, 62% injection site reactions, 36% hair loss, 16% depression, 3.5% lowered haemoglobin levels, 6% leukopenia, 27% lymphopenia, 23% thrombocytopenia, and 9% increased hepatic enzymes.

On the other hand, GA do not induce important side effects. The most common one is the so-called patterned reaction, a transient and self limiting combination of flushing, chest tightness, sweating, palpitation and anxiety, unpredictably occurring within minutes after injection and spontaneously resolving in less than 30 minutes. In RCTs this reaction occurred in 24% of patients [45]. Other side effects were dizziness in 66%, palpitations in 12%, dyspnoea in 12%, anxiety in 9%, faintness in 17%, cramps in 17%, nausea in

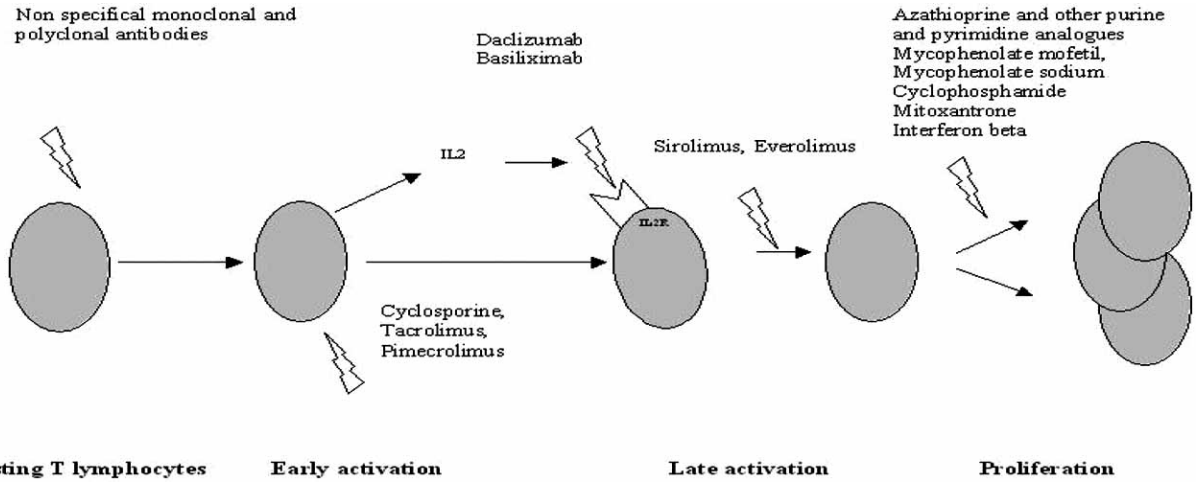

Fig. (3). Sites of action of commonly used immunosuppressants.

18%. Injection site reactions included itching in 47%, swelling in 48%, redness in 66%, and pain in 73%. Side effects induced treatment withdrawal only in 4%. It is very difficult to state with certainty which drug is best tolerated, in the absence of studies devoted to this issue.

Whether Aza induces an increased risk of cancer, as an effect of reduced immunosurveillance, is still a debated matter. Amato *et al*. found no increased risk of malignancies in 207 MS patients treated with Aza (mean time of 4.16 years and mean follow-up of 5.93 years) as compared to 247 untreated patients (mean follow-up of 6.73 years) [54]. Confavreaux *et al.* [55], using data from the Lyon Multiple Sclerosis Database (1,191 MS patients), analysed the relation between Aza treatment and cancer through a case-control study. The overall odds ratio was 1.7, not statistically significant. Nevertheless, the risk increased with longer duration of treatment associated with higher cumulative dose; the odds ratio was 1.3 in subjects treated for less than 5 years (less than 300 grams of cumulative dose), 2.0 when treated for 5 to 10 years (300-600 grams cumulative dose), and 4.4 when treated for more than 10 years (above 600 grams cumulative dose); however, the trend did not reach statistical significance. The types of cancer observed matched those seen in the general population, and, in addition, cancers usually reported in immunosuppressed patients (non-Hodgkin's lymphoma, squamous cell skin carcinoma, vulvar and perineal carcinoma, Kaposi's sarcoma and *in situ* cervical carcinoma) were not significantly increased. The Authors infer that an increased risk is present only after 10 years of continuous treatment or above 600 grams of cumulative dose. The mortality data from the largest RCT of azathioprine in MS [56] (British and Dutch MS Aza Trial Group [24]) showed a small but not significant increase in cancer and death in subjects tacking Aza than in ones taking placebo (increase in risk from a 3-years course of Aza: 3.4%, 95% CI –2.1, 9.0).

Data about the risk of cancer following treatment with immunomodulatory drugs are lacking. In a cohort of 1,338 MS patients in Israel [57], a reduced risk of malignancies was observed in MS female untreated patients, a finding tentatively explained by a better immunosurveillance due to MS-induced immune hyperactivity. After the beginning of immunomodulatory drugs, a non significant increased risk was observed, in particular for breast cancer in GA-treated patients, and other types of cancer in IFNB-treated female patients, thus questioning a role for immunomodulators in inducing cancer.

METHOTREXATE, CYCLOPHOSPHAMIDE, AND MITOXANTRONE IN MS TREATMENT

Methotrexate (MTX) is a potent oral immunosuppressant, whose mode of action involves the inhibition of dihydrofolate reductase, in addition to unspecific anti-inflammatory effects. A Cochrane review analysed the possible role of this drug in MS therapy [58], although very few RCTs are available. In 1993, a trial involving 20 RR patients randomized to MTX (9) or placebo (11) showed a tendency towards a reduced relapse risk in treated patients, without statistical significance [59]. The relative risk *vs*. placebo of one or more exacerbation was 0.35, but 95% CI was very large (0.10-1.28). The study was excluded from the Cochrane review for doubts about blindness. In 1995, Goodkin *et al*. randomized to MTX (31 patients) or placebo (29 patients) patients with progressive forms of MS [60]. There were no differences in the proportions remaining relapse free (27/31 MTX versus 24/29 placebo), although the population studied was at low risk of relapse from the outset. Eleven/31 methotrexate versus 15/29 placebo had sustained EDSS progression, without statistically significant differences.

Cyclophosphamide (CFX) is an alkylating agent with cytotoxic and immunosuppressive effects, used in the treatment of different malignancies as well as autoimmune diseases (Wegener's granulomatosis, periarteritis nodosa, systemic lupus erythematosus). CFX has also been reported to reduce the severity and prevent experimental allergic encephalomyelitis. Different treatment schedules have been adopted in MS, including varying dosages, route of administration (i.e. oral, intravenous), duration (ranging from a few days to months) and association with other drugs. The 2007 Cochrane meta-analysis [61] of RCTs identified just 2 studies comparing efficacy of CFX with placebo [62,63]. Both

trials involved only progressive forms, with or without superimposed relapses. A total number of 77 patients were treated, *vs*. 74 who took placebo. The relative risk of worsening (≥ 1.0 EDSS point if the basal point was ≤ 5.5, ≥ 0.5 if basal ≥ 6.0) for CFX *vs*. placebo was 0.92 at 1 year, 0.87 at 18 months, 1.05 at 24 months. The mean EDSS change difference (CFX *vs*. placebo) was -0.21 at 12 months, -0.19 at 18 months, 0.14 at 24 months. CFX treatment induced alopecia in 100% of patients, nausea and vomiting in 77%, amenorrhea in 42% (24% permanent), major infections in 11%, and cystitis in 4%. Four deaths occurred in the treated group. To date, the low efficacy of CFX in preventing disability progression in addition to a significant toxicity questions the role of CFX in MS therapy.

Mitoxantrone (MX) is a cytotoxic agent of the anthracenedione family, widely used for the treatment of breast cancer and leukaemias. MX acts by intercalating with DNA and inhibiting topoisomerase II. MX reduces the number of B cells, inhibits T helper cell function, and augments T cell suppressor activity. Its use in MS patients began after experimental findings in experimental allergic encephalomyelitis [64]. Results of 4 RCTs, comparing the efficacy of MX with placebo in MS have been published [65-68], and reviewed in a Cochrane meta-analysis [69]. The total number of participants was 270, 139 assigned to MX, 131 to placebo. These studies included both RR MS and progressive MS patients, with an important disability progression over the last year. Different dosage and time schedules were used, with MX being administered intravenously at cycles with intervals varying from one to three months. The relative risk of worsening (≥ 1.0 EDSS point if the basal point was ≤ 5.5, ≥ 0.5 if basal ≥ 6.0) for MX *vs*. placebo was 0.25 at 1 year, and 0.34 at 2 years. The mean EDSS change difference of MX *vs*. placebo was –0.35 at 1 year and –0.36 at 2 years. The relative risk *vs*. placebo of one or more exacerbations was 0.44 at 1 year, and 0.63 at 2 years. This efficacy in reducing disease progression and relapse risk must be evaluated, also in consideration of relevant side effects, including cardiotoxicity and leukaemia. Cardiotoxicity is secondary to hearth drug storage that causes a reduction of left ventricular ejection fraction in 3.6% of treated subjects. The cardiotoxicity represents the major limitation for long-term administration at a maximum cumulative dose of 140 mg/m^2, roughly corresponding to about two years of treatment. On the contrary, MX-related acute leukaemia is characterized by short latency, acute onset and a good response to therapy. A meta-analysis investigation on the incidence of leukaemia in a cohort of 1,378 MX-treated MS patients revealed an incidence of 0.25%, higher than the proportion of de novo leukaemia occurring in healthy patients (ranging from 0.001% at 20 years of age to 0.03 at 70 years) [70]. Other side effects of MX include amenorrhea in 26% of treated female (7.8% persisting after the end of therapy), nausea and vomiting in 62%, alopecia in 47%, urinary tract infections in 25%, respiratory tract infections in 35%, phlebitis in 14%, leukopenia in 13%, anemia in 7% and increased liver enzymes in 17%. Although MX represents one of the most effective therapy available at the present time, the important side effects, the risk of leukaemia and the cardiotoxicity make MX a drug with a restricted indication in patients with rapid disability progression.

NEW DRUGS: NATALIZUMAB AND FINGOLIMOD

Natalizumab is a selective adhesion-molecule inhibitor, that binds to the α_4 subunit of $\alpha_4\beta_1$ integrin on the surface of lymphocytes and blocks their binding to endothelial receptors, thereby impairing cell locomotion through the blood-brain barrier. Two RCTs on natalizumab in RR MS were published in 2006. The first compared natalizumab to placebo [71], the other assessed the efficacy of natalizumab plus IFNB in comparison to IFNB alone [72]. The placebo controlled trial involved 627 subjects in the natalizumab group and 315 in the placebo group. The relative risk in treated patients *vs*. placebo of one or more exacerbation was 0.50 (95% CI 0.41-0.62) at 1 year, and 0.51 (95% CI 0.44-0.61) at 2 years. The mean change *vs*. placebo of EDSS was not reported by the study (probably not statistically significant). The relative risk of worsening (≥ 1.0 EDSS point if the basal EDSS was ≤ 5.5, ≥ 0.5 if basal ≥ 6.0) in natalizumab-treated group was 0.29 (95% CI 0.16, 0.53), although it must be observed that also in the placebo group the risk of worsening was very low, about 10%. Side effects in these MS patients were headache, fatigue, arthralgia, urinary infections and respiratory infections, depression, rush and menstrual alterations. This new drug shows, therefore, a greater effect than older ones in reducing relapse rates. However, three cases of progressive multifocal leukoencephalopathy (PML) have been reported in natalizumab plus INFB-treated patients with MS or Chron's disease. For this reason, unless reassuring data will come from the post-marketing studies, the worry about the risk of PML remains and natalizumab is currently indicated only for MS patients with frequent relapses, in whom other treatments are believed to be uneffective.

Fingolimod is an oral sphingosine-1-phosphate receptor modulator. Its mechanism of action consists in depriving lymphocytes of a signal necessary to egress from secondary lymphoid tissues, therefore, sequestering them in the lymph nodes. In this way, the recirculation of lymphocytes in the central nervous system is reduced. The RCT on fingolimod involved 227 RR patients (92 assuming placebo, 93 assuming fingolimod at the dose of 1.25 mg once daily, 92 fingolimod 5.0 mg daily) with a follow-up of 6 months [73]. At the end of follow-up, the relative risk of having one or more relapses was 0.27 in the group treated with 1.25 mg *vs*. placebo, and 0.23 in the group treated with 5.0 mg *vs*. placebo. The relative risk for worsening was 0.48 in the 1.25 mg group *vs*. placebo, and 0.73 in the 5.0 group *vs*. placebo. No major effects on disability were observed. Even though promising, these results need to be confirmed in larger studies with a longer follow-up period.

AZA IN ANNO DOMINI 2008: CONCLUDING REMARKS

Although the usefulness of new therapies has been confirmed, the ideal drug for MS is still lacking. The largely used IFNB and GA are safe and well tolerated, but their effectiveness is limited to reducing the relapse risk in RR MS only during the first two years of treatment, while these drugs show little advantage on disability progression. On the other hand, MX benefit on disability is more evident, but toxicity limits its use in severe cases. Data on other immunosuppressive drugs, such as MTX and CFX, are disappoint-

ing, and, in addition, doubts exist on their effectiveness. Finally, the new drugs natalizumab and fingolimod need to be further evaluated, especially in regard to their efficacy and safety.

For all these reasons, we believe that an old drug such as Aza, maintains its importance in the "2008 therapy of MS". We must here recall that MS is a generally progressive, chronic disease of young people, and that, therefore, therapies must be tailored for long periods. Based on studies in the pre- and post-interferon era, Aza seems a valid alternative to IFNB or GA. In addition, Aza can be used also in patients (i) who do not tolerate IFNB side effects, (ii) who reject frequent subcoutaneous injections, or (iii) who develop non-response to these drugs. Notably, a better impact on the quality of life was observed for Aza-treated patients in a small trial [52], and it would be important to confirm this result in a larger RCT. A major pitfall in the treatment of MS is the low efficacy of all available therapies in preventing disability progression. Only MX showed a significant benefit in reducing EDSS worsening *vs*. placebo, although this gain is counterbalanced by an increased risk of heart dysfunction of leukaemia. MX is currently indicated for subjects with a high risk of disability, for a limited two-year period. However, the question remains how to treat subjects who have reached the maximum MX cumulative dose. We here suggest that in such cases Aza remains a valid alternative for continuing therapy. For all the aforementioned reasons, we think that Aza is still, in 2008, an useful therapeutic option in the management of MS.

ABBREVIATIONS

6-MMP	=	6-methylmercaptopurine
6-MP	=	6-mercaptopurine
6-TGN	=	6-thioguanine –nucleotides
6-thioGTP	=	6-thioguanine triphosphate
Aza	=	azathioprine
BBB	=	Blood-brain-barrier
CFX	=	Cyclophosphamide
CNS	=	Central nervous system
EDSS	=	Expanded disability status score
GA	=	Glatiramer acetate
HGPRT	=	hypoxanthine guanine phosphoribolsyltransferase
INFB	=	Interferon beta
MRI	=	Magnetic resonance imaging
MS	=	Multiple sclerosis
MTX	=	Methotrexate
MX	=	Mitoxantrone
PML	=	Progressive multifocal leukoencephalopathy
QALY	=	Quality-adjusted life years
RCT	=	Randomized clinical trial
RR	=	Relapsing-remitting
TPMT	=	thiopurine methyltransferase

REFERENCES

[1] Frohman, E.M.; Racke, M.K.; Raine, C.S. *N. Engl. J. Med.*, **2006**, *354*, 942.
[2] Hauser, L.H.; Oksenberg, J.R. *Neuron,* **2006**, *52*, 61.
[3] Kutzelnigg, A.; Lucchinetti, C.F.; Stadelmann, C.: Bruck, W.: Rauschka, H.; Bergmann, M.; Schmidbauer, M.; Parisi, J.E.; Lassmann, H. *Brain,* **2005**, *128*, 2705.
[4] Zarei, M. *J. Neurol. Sci*., **2006**, *245,* 53.
[5] Confavreux, C.; Vukusic, S.; Moreau, T. ; Adeleine, P. *N. Engl. J. Med.,* **2000**, *343*, 1430.
[6] Gardiner, S.J.; Begg, E.J. *Pharmacol. Rev.,* **2006**, *58*, 521.
[7] Otterness, D.; Szumlanski, C.; Lennard, L.; Klemetsdal, B.; Aarbakke, J.; Park-Hah, J.O.; Iven, H.; Schmiegelow, K.; Branum, E.; O'Brien, J.; Weinshilboum, R.M. *Clin. Pharmacol. Ther*., **1997**, *62*, 60.
[8] Otterness, D.; Szumlanski, C.; Wood, T.C.; Weinshilboum, R.M. *J. Clin. Investig.,* **1998**, *101*, 1036.
[9] Gardiner, S.J.; Begg, E.J. *Pharmacogenet. Genomics*, **2005**, *15*, 365.
[10] Siegel, C.A.; Sands, B.E. *Aliment. Pharmacol. Ther.,* **2005**, *22*, 1.
[11] Colombel, J.F.; Ferrari, N.; Debuysere, H.; Marteau, P.; Gendre, G.P.; Bonaz, B.; Soule, J.C.; Modigliani, R.; Touze, Y.; Catala, P.; Libersa, C.; Broly, F. *Gastroenterology,* **2000**, *118*, 1025.
[12] Nygaard, U.; Toft, T.; Schmiegelow K. *Clin. Pharamcol. Ther.,* **2004**, *75*, 274.
[13] Suthanthiran, M.; Strom, T.B. *Pediatr. Nephrol.,* **1997**, *11*, 651.
[14] Tiede I.; Fritz G.; Strand S.; Poppe D.; Dvorsky R.; Strand D.; Lehr H.A.; Wirtz S.; Becker C.; Atreya R.; Mudter J.; Hildner K.; Bartsch B.; Holtmann M.; Blumberg R.; Walczak H.; Iven H.; Galle P.R.; Ahmadian M.R.; Neurath M.F. *J. Clin. Invest.,* **2003**, *111*, 1133.
[15] Younger, D. S. *Curr. Opin. Neurol.,* **2004**, *17*, 317.
[16] Monaco, S.; Turri, E.; Zanusso, G.; Maistrello, B. *Curr. Drug Targets Immune Endocr. Metabol. Disord.,* **2004**, *4*,141.
[17] Allison, A.C. *Immunopharmacology*, **2000**, *47*, 63.
[18] Noseworthy, J.H. *Nature,* **1999**, *399*, 40.
[19] IFNB MS Study Group. *Neurology,* **1993**, *43*, 655.
[20] Lublin, F.D.; Reingold, S.C. *Neurology,* **1996**, *46*, 907.
[21] Yudkin, P.L.; Ellison, G.W.; Ghezzi, A.; Goodkin, D.E.; Hughes, R.A.C.; McPherson, K.; Mertin, J.; Milanese, C. *Lancet,* **1991**, *26*, 1051.
[22] Swinburn, W.R.; Liversedge, L.A. *J. Neurol. Nerurosurg. Psychiatry,* **1973**, *36*, 124.
[23] Mertin, J.; Rudge, P.; Kremer, M.; Healey, M.J.; Knight, S.C.; Compston, A.; Batchelor, J.R.; Thompson, E.J.; Halliday, A.M.; Denman, M.; Medawar, P.B. *Lancet,* **1982**, *2*, 351.
[24] British and Dutch Multiple Sclerosis Azathioprine Trial Group. *Lancet,* **1988**, *2*, 179.
[25] Milanese, C.; La Mantia, L.; Salmaggi, A.; Campi, A.; Bortolami, C.; Tajoli, L.; Nespolo, A.; Corridori, F. *Ital. J. Neurol. Sci.,* **1988**, *9*, 53.
[26] Ghezzi, A.; Di Falco, M.; Locatelli, C.; Zaffaroni, M.; Caputo, D.; Marfono, S. In *Recent Advances in Multiple Sclerosis Therapy*; Gonsette, R.E.; Delmotte, P., Eds.; Elsevier Science B.V.; Amsterdam, **1989**; pp. 345-6.
[27] Eleison, G.W.; Myers, L.W.; Mickey, R.; Graves, M.C.; Tourtellotte, W.W.; Syndulko, K.; Holevoet-Howson, M.I.; Lerner, C.D.; Frane, M.V.; Pettler-Jennings, P. *Neurology,* **1989**, *39*, 1018.
[28] Goodkin, D.E.; Bailly, R.C.; Teetzen, M.L.; Hertsgaard, D.; Beatty, W.W. *Neurology,* **1991**, *41*, 20.
[29] Kurtzke, J.F. *Neurology,* **1983**, *33*, 1444.
[30] Cavazzuti, M.; Merelli, E.; Tassone, G.; Mavilla, L. *Eur. Neurol.,* **1997**, *38*, 284.
[31] Massacesi, L.; Parigi, A.; Barilaro, A.; Repice, A.M.; Pellicanò, G.; Konze, A.; Siracusa, G.; Taiuti, R.; Amaducci, L. *Arch. Neurol.,* **2005**, *62*, 1843.
[32] Jacobs, L.D.; Cookfair, D.L.; Rudick, R.A.; Herndon, R.M.; Richert, J.R.; Salazar, A.M.; Fisher, J.S.; Granger, C.V.; Simon,

J.H.; Alam, J.J.; Bartoszak, D.M.; Bourdette, D.N.; Braiman, J.; Brownschiedle, C.M.; Coats, M.E.; Cohan, S.L.; Dougherty, D.S.; Kinkel, R.P.; Mass, M.K.; Munschauer, F.E. 3rd; Priore, R.L.; Pullicino, P.M.; Scherokman, B.J.; Whitham, R.H.; The Multiple Sclerosis Collaborative Research Group (MSCRG). *Ann. Neurol.,* **1996**, *39*, 285.

[33] PRISMS Study Group. *Lancet,* **1998**, *352*, 1498.

[34] Paty, D.W.; Li, D.K. *Neurology,* **1993**, *43*, 662.

[35] Rice, G.P.A.; Incorvaia, B.; Munari, L.; Ebers, G.; Polman, C.; D'Amico, R.; Filippini, G. *Cochrane Database Systematic Rev.,* **2001**, *Issue 4.*

[36] Knobler, R.L.; Greenstein, J.I.; Johnson, K.P.; Lublin, F.D.; Panitch, H.S.; Conway, K.; Grant-Gorsen, S.V.; Muldoon, J.; Marcus, S.G.; Wallenberg, J.C. *J. Interferon. Res.,* **1993**, *13*, 333.

[37] Durelli, L.; Bongioanni, M.R.; Cavallo, R.; Ferrero, B.; Ferri, R.; Ferrio, M.F.; Bradac, G.B.; Riva, A.; Vai, S.; Geuna, M.; Bergamini, L.; Bergamasco, B. *Neurology,* **1994**, *44*, 406.

[38] The PRISMS (Prevention of Relapses and Disability by Interferon beta-1a Subcutaneously in Multiple Sclerosis) Study Group. *Neurology,* **2001**, *56*, 1628.

[39] Myhr, K.M.; Riise, T.; Green Lilleas, F.E.; Beiske, T.G.; Celius, E.G.; Edland, A.; Jensen, D.; Larsen, J.P.; Nilsen, R.; Nortvedt, M.W.; Smievoll, A.I.; Vedeler, C.; Nyland, H.I. *Neurology,* **1999**, *52*, 1049.

[40] The Once Weekly Interferon for MS Study Group (OWIMS). *Neurology,* **1999**, *53*, 679

[41] Stone, L.A.; Frank, J.A.; Albert, P.S.; Bash, C.; Smith, M.E.; Maloni, H.; McFarland, H.F. *Ann. Neurol.,* **1995**, *37*, 611.

[42] Pozzilli, C.; Bastianello, S.; Koudriavtseva, T.; Gasperini, C.; Bozzao, A.; Millefiorini, E.; Galgani, S.; Buttinelli, C.; Perciaccante, G.; Piazza, G.; Bozzao, L.; Fieschi, C. *J. Neurol. Neurosurg. Psychiatry,* **1996**, *61*, 251.

[43] Waubant, E.; Goodkin, D.E.; Sloan, R.; Andersson, P.B. *Neurology,* **1999**, *53*, 874.

[44] Teitelbaum, D.; Arnon, R.; Sela, M. *Cell. Mol. Life Sci.,* **1997**, *53*, 24.

[45] Johnson, K.P.; Brooks, B.R.; Cohen, J.A.; Ford, C.C.; Goldstein, J.; Lisak, R.P.; Myers, L.W.; Panitch, H.S.; Rose, J.W.; Schiffer, R.B. *Neurology,* **1995**, *45*, 1268.

[46] Munari, L.; Lovati, R.; Boiko, A. *Cochrane Database Sys. Rev.,* **2003**, *Issue 4.*

[47] Bornstein, M.B.; Miller, A.; Slagle, S.; Weitzman, M.; Crystal, H.; Drexler, E.; Keilson, M.; Merriam, A.; Wassertheil-Smoller, S.; Spada, V.; Weiss, W.; Arnon, R.; Jacobsohn, I; Teitelbaum, D.; Sela, M. *N. Engl. J. Med.,* **1987**, *317*, 408.

[48] Comi, G.; Filippi, M.; Wolinsky, J.S. and the European/Canadian Glatiramer Acetate Study Group. *Ann. Neurol.,* **2001**, *149*, 290.

[49] Palace, J.; Rothwell, P. *Lancet,* **1997**, *350*, 261.

[50] Sudlow, C.L.; Counsell, C.E. *BMJ,* **2003**, *326*, 388.

[51] Rubio-Terrés, C.; Domínguez-Gil Hurlé. A. *Rev. Neurol. (Madrid),* **2005**, *40*, 705.

[52] Milanese, C.; La Mantia, L.; Salmeggi, A. *J. Neurol. Neurosurg. Psychiatry,* **2001**, *70*, 413.

[53] Aimard, G.; Confavreaux, C.; Ventre, J.J.; Guillot, M.; Devic, M. *Rev. Neurol. (Paris),* **1983**, *139*, 509.

[54] Amato, M.P.; Pracucci, G.; Ponziani, G.; Siracusa, G.; Fratiglioni, L.; Amaducci, L. *Neurology,* **1993**, *43*, 831.

[55] Confavreaux, C.; Saddier, P.; Grimaud, J.; Moreau. T.; Adeleine, P.; Aimard, G. *Neurology,* **1996**, *46*, 1607.

[56] Taylor, L.; Hughes, R.A.C.; McPherson, K. *Eur. J. Neurol.,* **2004**, *11*, 141.

[57] Achiron, A.; Barak, Y.; Gail, M.; Mandel, M.; Pee, D.; Ayyagari, R.; Rotstein, Z. *Breast Cancer Res. Treat.,* **2005**, *89*, 265.

[58] Gray, O.; McDonnel, G.V.; Forbes, R.B. *Cochrane Database Sys. Rev.,* **2004**, *Issue 2.*

[59] Currier, R.D.; Hearer, A.F.; Meydrech, E.F. *J. Neurol. Neurosurg. Psychiatry,* **1993**, *56*, 1217.

[60] Goodkin, D.E.; Rudick, R.A.; Vander-Brug Medendorp, S.; Daughtry, M.M.; Schwetz, K.M. *Ann. Neurol.,* **1995**, *37*, 30.

[61] La Mantia, L.; Milanese, C.; Mascoli, N.; D'Amico, R.; Weinstock-Guttman. B. *Cochrane Database Sys. Rev.,* **2007**, *Issue 1.*

[62] Likosky, W.H.; Fireman, B.; Elmore, R.; Eno, G.; Gale, K.; Goode, B.G.*J. Neurol. Neurosurg. Psychiatry,* **1991**, *54*, 1055.

[63] The Canadian Cooperative Multiple Sclerosis Study Group, Noseworthy, J.H.; Ebers, G.C.; Gent, M.; Seland, T.P.; Shumak, K.H. *Lancet,* **1991**, *337*, 441.

[64] Lublin, F.D.; Lavasa, M.; Viti, C.; Knobler, R.L. *Clin. Immunol. Immunopathol.,* **1987**, *45*, 122.

[65] Edan G, Miller D, Clanet M, Confavreux C, Lyon-Caen O, Lubetzki C, Brochet B, Berry I, Rolland Y, Froment JC, Cabanis E, Iba-Zizen MT, Gandon JM, Lai HM, Moseley I, Sabouraud O. *J. Neurol. Neurosurg. Psychiatry,* **1997**, *62*, 112.

[66] Hartung, H.P.; Gonsette, R.; Konig, N.; Kwiecinski, H.; Guseo, A.; Morrissey, S.P. *Lancet,* **2002**, *360*, 2018.

[67] Millefiorini, E.; Gasperini, C.; Pozzilli, C.; D'Andrea, F.; Bastianello, S.; Trojano, M. *J. Neurol.,* **1997**, *244*, 153.

[68] van deWyngaert, F.A.; Beguin, C.; D'Hooghe, M.B.; Dooms, G.; Lissoir, F.; Carton, H.; Sindic, C.J. *Acta Neurol. Belgica,* **2001**, *101*, 210.

[69] Martinelli Boneschi, F.; Rovaris, M.; Capra, M.; Comi, G. *Cochrane Database Sys. Rev.,* **2005**, *Issue 4.*

[70] Ghalie, R.G.; Mauch, E.; Edan, G.; Hartung, H.P.; Gonsette, R.E.; Eisenmann, S. *Multiple Sclerosis,* **2002**, *8*, 441.

[71] Polman, C.H.; O'Connor, P.W.; Havrdova, E.; Hutchinson, M.; Kappos, L.; Miller, D.H.; Phillips, J.T.; Lublin, F.D.; Giovannoni, G.; Wajgt, A.; Toal, M.; Lynn, F.; Panzara, M.A.; Sandrock, A.W. *N. Engl. J. Med.,* **2006**, *354*, 899.

[72] Rudick, R.A.; Stuart, W.H.; Calabresi, P.A.; Confavreux, C.; Galetta, S.L.; Radue, E-W.; Lublin, F.D.; Weinstock-Guttman, B.; Wynn, D.R.; Lynn, F.; Panzara, M.A.; Sandrock, A.W.; for the SENTINEL Investigators. *N. Engl. J. Med.,* **2006**, *354*, 911.

[73] Kappos, L.; Antel, J.; Comi, G.; Montalban, X.; O'Connor, P.; Polman, C.H.; Haas, T.; Korn, A.A.; Karlson, G.; Radue, E.W.; for the FTY720 D2201 Study Group. *N. Engl. J. Med.,* **2006**, *355*, 1124.

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