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Cyclophosphamide in multiple sclerosis: scientific rationale, history and novel treatment paradigms

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***Abstract*:** For patients with relapsing–remitting multiple sclerosis (RRMS), there are currently six approved medications that have been shown to alter the natural course of the disease. The approved medications include three beta interferon formulations, glatiramer acetate, natalizumab and mitoxantrone. Treating aggressive forms of RRMS and progressive disease forms of MS still presents a great challenge to neurologists. Intense immunosuppression has long been thought to be the only feasible therapeutic option. In patients with progressive forms of MS, lymphoid tissues have been detected in the central nervous system (CNS) that may play a critical role in perpetuating local inflammation. Agents that are currently approved for patients with MS have no or very limited bioavailability in the brain and spinal cord. In contrast, cyclophosphamide (CYC), an alkylating agent, penetrates the blood–brain barrier and CNS parenchyma well. However, while CYC has been used in clinical trials and off-label in clinical practice in patients with MS for over three decades, data on its efficacy in very heterogeneous groups of study patients have been conflicting. New myeloablative treatment paradigms with CYC may provide a therapeutic option in patients that do not respond to other agents. In this article we review the scientific rationale that led to the initial clinical trials with CYC. We will also outline the safety, tolerability and efficacy of CYC and provide neurologists with guidelines for its use in patients with MS and other inflammatory disorders of the CNS, including neuromyelitis optica (NMO). Finally, an outlook into relatively novel treatment approaches is provided.

***Keywords*:** multiple sclerosis, MS, Cytoxan, cyclophosphamide, treatment, therapy, immunology

Cyclophosphamide

Mechanism of action

Cyclophosphamide (CYC) is an alkylating chemotherapeutic agent related to nitrogen mustard that binds to DNA and interferes with mitosis and cell replication [Kovarsky, 1983]. CYC targets predominantly rapidly dividing cells, and it has been widely used as an antineoplastic medication in a range of solid tumors and hematological malignancies. It is FDA-approved to be used in the treatment of lymphomas, leukemias, multiple myeloma, breast carcinoma, ovarian adenocarcinoma, retinoblastoma, disseminated neuroblastoma, advanced mycosis fungoides and minimal change nephritic syndrome in children [ONS Clinical Practice Committee].

In addition to the antimitotic and antireplicative effects CYC has immunosuppressive as well as immunomodulatory properties. Most of these properties were discovered many years after the drug was first utilized in patients with autoimmune diseases. Specifically, it causes suppression of cell-mediated and humoral immunity through its actions on T cells and B cells [Kim *et al.* 2009]. In multiple sclerosis (MS), CYC has been shown to decrease the secretion of the pro-inflammatory T helper (Th) 1 cytokine interferon-γ (IFNγ) and interleukin (IL)-12 and to increase the secretion of the anti-inflammatory Th2 cytokines IL-4 and IL-10 in cerebrospinal fluid (CSF) and peripheral blood [Smith *et al.* 1997]. Increased peripheral secretion of IFNγ and IL-12 has been reported in patients with

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secondary progressive MS (SPMS) *in vitro* [Comabella *et al.* 1998; Balshov *et al.* 1997]. Interestingly, in these studies increased concentrations of both cytokines were shown to correlate with clinical disease activity [Comabella *et al.* 1998; Balshov *et al.* 1997]. Specifically, elevated levels of IFNγ preceded clinical attacks [Comabella *et al.* 1998; Balshov *et al.* 1997].

CYC therapy also appears to alter T lymphocytes towards a less inflammatory phenotype. During MS relapses, $CXCR3^+$ and $CCR5^+$ IFNγ-producing T cells were readily detectable in the CSF of MS patients [Karni *et al.* 2004; Takashim *et al.* 1998]. The frequency of these cells also appears to increase in patients with SPMS in the peripheral blood [Karni *et al.* 2004; Takashim *et al.* 1998]. In one of these studies, CYC therapy upregulated the percentage of anti-inflammatory $CCR4^+$ IL-4-producing T cells and normalized the percentages of the $CCR5^+$ and $CXCR3^+$ T cells in patients with SPMS [Karni *et al.* 2004]. The induction of a Th2-type response by CYC was reproduced in several studies and appears to positively affect the course of MS [Karni *et al.* 2004; Takashim *et al.* 1998]. In a study by Gladstone and co-workers [2007], high-dose CYC resulted in cellular profiles similar to the ones achieved by bone marrow transplant, including the preferential targeting of naïve T cells. In this particular study, CYC therapy was also associated with diminished clinical disease activity.

With chronicity of MS, the inflammatory response becomes trapped behind the blood–brain barrier (BBB), giving rise to slowly progressive inflammatory damage that affects the brain and spinal cord [Steinman, 2001]. This process seems to be driven by the aberrant formation of ectopic lymphatic tissue within the brain compartment [Prineas, 1979; Prineas and Wright, 1978]. It has to be assumed that most pharmacotherapies that are currently approved for MS or are in clinical development do not penetrate the BBB. In addition, these agents may have limited indirect effects on immunological events within the CNS. For instance, natalizumab may prevent most of B cells and plasma cells from entering the brain and spinal cord, but it has no effect on the number and quantity of oligoclonal bands (OCBs), total IgG, or IgG synthesis in the CSF [Stuve *et al.* 2009, 2006]. The B cell-depleting antibody rituximab also has no effect on OCBs in the CSF [Cross *et al.* 2006]. Thus, it has to be assumed that potentially pathogenic plasma cells and other immune cells may persist in the CNS compartment despite these aggressive treatment approaches.

In contrast, CYC has the advantage of good bioavailability within the CNS. Consequently, it may induce local immunomodulation and immunosuppression even after the formation of lymphatic tissues in the brain and spinal cord that could potentially result in stabilizing the disease and preventing further progression. Proof of principle for the direct intrathecal and intracerebral actions of CYC on compartmentalized immune cells was provided in a disease other than MS. Kanter *et al.* [2008] reported a case of anti-glutamic acid decarboxylase (GAD) antibody associated refractory status epilepticus where treatment with CYC resulted in cessation of seizures and intrathecal synthesis of anti-GAD antibody [Kanter *et al.* 2008]. Given that all currently approved agents for patients with RRMS do not penetrate the BBB (with the exception of mitoxantrone), CYC may potentially play a therapeutic role in patients with advanced disease. This potential has not been explored in a controlled setting of a state-of-the-art randomized clinical trial that enrols patients according to modern diagnostic criteria [Polman *et al.* 2005]. In addition, novel imaging modalities have not been applied to determine the effects of CYC on CNS demyelination and neurodegeneration.

Safety and tolerability of cyclophosphamide in MS patients

The major safety issues that have been associated with CYC include bladder cancer and gonadal toxicity. These will be discussed in separate sections. Common side effects of CYC in the clinical trials include: alopecia, nausea/vomiting, transient myelosuppression, hemorrhagic cystitis, amenorrhea and transient azospermia [Perini *et al.* 2007; Compendium of Pharmaceuticals and Specialties, 2006; Portaccio *et al.* 2003; Hohol *et al.* 2009; Weinstock-Guttman 1997; Talar-Williams *et al.* 1996; Likosky *et al.* 1991].

In 2003, Portaccio and co-workers [2003] evaluated 112 patients with MS who received pulse CYC of 700 mg/m^2 monthly for 12 months then bimonthly for 12 months and found that 81.8% of patients considered the treatment tolerable. Discontinuation of treatment due to serious side effects occurred in 18% of patients. Serious side effects happened in 21.4% of patients and

included: amenorrhea (33.3% of fertile women), hypogammaglobulinemia (5.4%), hemorrhagic cystitis (4.5%) and malignancies (3.6%). Malignancies were seen in four patients and three of them were treated with azathioprine before CYC.

Perini and co-workers [2007] evaluated the safety profile of CYC in MS patients in 2007. They concluded that CYC, using the usual MS protocol (monthly for 1 year then bimonthly for extra year), is well tolerated. The most common side effects were mild alopecia, nausea, vomiting and cystitis and these side effects were transient and completely reversible. Amenorrhea was only observed in older women (more than 40 years of age).

Cyclophosphamide-induced hemorrhagic cystitis

As already mentioned, hemorrhagic cystitis can be seen in up to 4.5% of MS patients treated with CYC [Kanter *et al.* 2008]. On the other hand, hemorrhagic cystitis can be seen in 10% of cancer patients treated with CYC and more than 40% of patients treated with high-dose CYC (HDC) for bone marrow transplantation [Compendium of Pharmaceuticals and Specialties, 2006; Cross *et al.* 2006].

Increasing fluid intake and/or administering sodium 2-mercaptoethanesulfonate (mesna), a thiol compound, can reduce the risk of this complication [Takamoto *et al.* 2004]. Acrolein, the metabolite of cyclophosphamide, irritates mucous membranes and is considered pathogenetically important in hemorrhagic cystitis. Monitoring of urinary acrolein concentration could indicate when to take heightened preventive measures against hemorrhagic cystitis [Takamoto *et al.* 2004].

Bladder cancer in MS patients treated with cyclophosphamide

De Ridder and co-workers [1998] reported a retrospective study of 2351 patients with MS. Of the 2351 patients, two women and five men (0.29%) had bladder cancer. Of the 850 chronically catheterized patients, the incidence was 0.7%. In the subgroup of 70 patients treated with CYC, five chronically catheterized patients (5.7%) had bladder cancer. The mean time lapsed from the last dose of CYC until the occurrence of neoplastic growth was 5.8 years (range 3–10 years). No other CYC-induced tumors were detected.

While all of the aspects of CYC-associated oncogenesis remain incompletely understood, the involvement of nitric oxide is an almost universally accepted co-factor. The mechanism of nitric oxide carcinogenesis is twofold: DNA damage and cytotoxicity [Tamir and Tannenbaum, 1996]. Chronic urinary tract infection due to indwelling catheterization and exposure to high doses of CYC may have a synergistic effect on the carcinogenesis of bladder cancer. The author recommends annual cystoscopy in patients with MS with indwelling catheters, especially those who received CYC.

The exact incidence and prevalence of CYC-associated neoplasms have yet to be determined. For instance, Lebrun and co-investigators reported a descriptive study of MS patients with documented oncologic event in 2007 [Lebrun *et al.* 2008]. These investigators retrospectively gathered information on 7428 MS patients from nine French centers between 1995 and 2006 (after the era of immunosuppressive therapy) and found no increased risk of cancer in MS patients exposed to disease-modifying agents including CYC compared to the general population. These results contrast with results reported by De Ridder in 1998. Based on experience with other autoimmune diseases, long-term oral CYC has been associated with significant risk of bladder cancer [Talar-Williams *et al.* 1996; Moore, 1991], a finding that resulted in abandoning oral CYC in MS. The experience of Radis and co-workers shed some light on the safe accumulative dose of CYC [Radid *et al.* 1995]. They published a 20-year follow-up study in 119 patients with rheumatoid arthritis who received oral cyclophosphamide. The risk of malignancy appears to increase as a function of total dosage. Accumulative doses of more than 80–100 g are associated with the highest risk of malignancy.

Gonadotoxicity secondary to cyclophosphamide use in MS

Infertility is one of the major limiting factors concerning the use of CYC in young patients with aggressive forms of MS. Amenorrhea is a common side effect in women with childbearing period (CBP) and the risk ranges from 33.3–44.7% in MS patients treated with CYC [Kanter *et al.* 2008; Wetzels, 2004]. Extreme caution should be taken when starting

a patient in her CBP on CYC and should only be initiated if warranted and with the patient's full understanding and consent. Blumenfeld [2007] showed that the use of IM monthly gonadotropin-releasing hormone (GnRH) resulted in restoration of normal ovarian cycle in more than 93% of women exposed to gonadotoxic medications including CYC. Larger studies will be required to substantiate the *in vivo* effect of GnRH-a as an unequivocal means of minimizing follicular apoptosis.

In contrast to amenorrhea, azoospermia is clearly related to the dose of CYC. Doses more than 7.2 g/m^2 and accumulative doses more than 300 mg/kg carry the highest risk in non-MS patients and the onset can be early or late [Wetzels 2004]. Thus far, no studies have evaluated the risk of azoospermia in MS patients treated with CYC. For instance, it is currently unclear whether or not effects on spermatogenesis are reversible or permanent. Blake and co-workers reported the case of a man who developed azoospermia after CYC treatment that resolved after 3 years of treatment cessation [Blake *et al.* 1976]. Masala and co-workers [1997] conducted a randomized clinical trial of 15 patients with nephritic syndrome who were treated with CYC. Five patients were given testosterone 100 mg intramuscularly (IM) every 15 days. All patients developed oligospermia and azoospermia. The sperm count normalized after 6 months in the group that received testosterone therapy, but only in one patient in the control group. More insight into these potential adverse events and possible interventions is clearly needed before CYC can be considered as pharmacotherapy for a broad group of young patients with MS.

Pregnant patients with MS and cyclophosphamide

CYC is teratogenic in animals, but population studies have not conclusively demonstrated its teratogenicity in humans [Meirow *et al.* 2001; Ujházy, 1993]. Until more information regarding safety is available; CYC should not be used in pregnancy and should be discontinued before anticipated pregnancy.

Elderly patients with MS and cyclophosphamide

There are no clinical data to guide the use of CYC in elderly patients. Given complications associated with immunosenescence, a potent immunosuppressive agent like CYC should be used with caution in senior individuals.

Children with MS and cyclophosphamide

A recent multicenter retrospective study conducted by Makhani and co-workers [2009] evaluated the use of CYC in 17 children. The children in this study were diagnosed with aggressive MS (15 with RRMS and two with SPMS) refractory to first-line disease-modifying agents. The 17 children had a mean age at first CYC use of 15.0 years (range 9.1–18.4 years) and mean disease duration of 3.1 years (range 0.3–6.4 years). Ten children (59%) were girls and 7 (41%) were boys. The Expanded Disability Status Scale (EDSS) scores at treatment initiation were available for 16 children and mean EDSS was 3.7. Interestingly, four children had an EDSS of 6.0 or higher. Three different protocols were utilized: (1) induction alone, (2) induction plus maintenance, or (3) maintenance alone. CYC was administered at 600–1000 mg/m^2 per dose. EDSS scores; annual relapse rates before, during, and after treatment with CYC; and all reported side effects were determined. For each patient, all brain MRI scans obtained from 12 months before initiation of CYC and up to 12 months after treatment were reviewed.

All but one patient had experienced worsening of EDSS scores or multiple relapses prior to treatment initiation. After conclusion of CYC therapy, 7 (41%) children continued a single standard first-line therapy. One child was not on any disease-modifying agents 1 year after CYC. The remaining nine children (53%) required combination therapy or treatment with a second-line therapeutic agent.

CYC was well tolerated in most patients. However, side effects included vomiting, transient alopecia, osteoporosis and amenorrhea. One patient developed bladder cancer. The study suggests that for children with aggressive disease, CYC is a potential option. However, given the potential long-term side effects of CYC, the risk–benefit ratio should be considered even more carefully than in adult patients. Also, randomized, prospective trials of CYC in young patients with refractory disease are needed to clearly assess defined treatment outcomes.

Treatment paradigms with cyclophosphamide

Cyclophosphamide monotherapy in MS

For patients with RRMS, there are currently six approved medications that have been shown to

alter the natural course of the disease. The approved medications include three beta interferon (IFNβ) formulations (Avonex, Rebif and Betaseron), glatiramer acetate (GA; Copaxone), natalizumab (Tysabri) and mitoxantrone (Novantrone). There is currently no evidence that IFNβ, GA, and natalizumab are effective in patients with very aggressive forms of RRMS and progressive disease forms of MS. Mitoxantrone is approved for treatment of secondary progressive MS with worsening relapsing and progressive relapsing disease course. Mitoxantrone has several potentially very severe side effects and its lifetime cumulative dose is restricted by regulatory agencies to 140 mg/m^2.

Given that the outcome in this patient population is usually poor with profound impact on lifestyle and productivity, alternatives to these six approved agents are needed by clinical neurologists. Intense immunosuppression has long been thought to be the only feasible therapeutic option. Over the past three decades, substantial experience has been gathered with CYC.

The first study that evaluated the safety and efficacy of CYC in MS was an open-label trial conducted by Hommes and co-workers in 1975. This and subsequent trials with CYC as a potent immunosuppressant were not without controversy, given the perception at that time of MS as a viral or postviral disorder. Hommes *et al.* [1975] treated 86 patients with chronic progressive MS with a short course of 400 mg intravenous (IV) CYC in combination with 1 g prednisone daily. Approximately 69% of patients experienced clinical stabilization of their disease for a period of 1–5 years. The second noteworthy trial was conducted by Hauser and his coworkers in 1983. These investigators conducted another open-label, prospective, randomized trial in 58 patients with a clinical phenotype that was termed 'progressive MS'. Study patients were randomized into three treatment arms: (1) a short course of high-dose IV CYC with adrenocorticotropin hormone (ACTH), (2) low-dose IV CYC with ACTH in addition to plasma exchange (PLEX), or (3) ACTH alone. Stabilization of clinical disease was reported in 80%, 50% and 20% of the respective treatment arms. These results were interpreted as evidence for the beneficial effects of high-dose IV CYC in patients with progressive MS. In retrospect, the interpretation of this trial is complex due to the fact that diagnostic criteria for MS relevant for use in clinical trials were only introduced in 1983 by Poser *et al.* [1983]. Recognition of the substantial clinical and paraclinical differences between patients with RRMS, progressive-relapsing MS (PRMS), SPMS, and primary progressive MS (PPMS) occurred even later [Lublin and Reingold 1996] and are still not fully understood. It has to be assumed that the patients enrolled by Hauser *et al.* were very heterogeneous and may not have been appropriately randomized among treatment arms.

Conflicting data on the use of CYC in MS came from two large randomized trials between 1991 and 1993. The Northeast Cooperative Multiple Sclerosis Treatment Group conducted a randomized trial of 256 patients with progressive MS who were randomly assorted to one of four arms: (1) IV CYC and ACTH was administered according to an established induction regimen (600 mg/m^2 daily for 5 days), (2) with or without IV boosters of CYC (700 mg/m^2) every other month, or (3) IV CYC and ACTH given according to a modified regimen (600 mg/m^2 on days 1, 2, 4, 6 and 8), with or (4) without IV boosters of CYC (700 mg/m^2) every other month [Weiner *et al.* 1993]. Initially, there were no differences in disease stabilization between the two induction regimens. However, patients who received maintenance boosters of CYC experienced a significant delay in reaching time-to-treatment failure-defined as one point increase on the EDSS [Kurtzke, 1983] that lasted for 2 months compared with the group that did not receive CYC boosters. A significant therapeutic effect was more pronounced among younger individuals.

These encouraging results were challenged by the results of a clinical trial conducted by the Canadian Cooperative Multiple Sclerosis Group. The Canadian trial was a single-blinded, placebo-controlled, multicenter trial that randomly assigned 168 patients with progressive MS to one of three arms: (1) IV CYC with oral prednisone, (2) oral CYC and oral (PO) prednisone on alternate days with weekly PLEX, or (3) oral placebo with sham PLEX [Canadian Cooperative Multiple Sclerosis Study Group, 1991]. The investigators of this trial reported no significant differences in terms of time-to-treatment failure defined as a worsening of one or more points on the EDSS sore on two consecutive exams separated by a minimum of 6 months. The conflicting data from these two large trials may also have resulted from distinct

patient populations, and ultimately resulted in an ongoing debate about the efficacy of CYC in patients with MS. The regional influence of these two studies on the neurology community can perhaps best be appreciated by looking at the data reported by Oger in 2007. He reported that CYC is used relatively frequently in patients with MS in France and the US (in respectively 6.9% and 5.5% of patients), whereas Canadian, British, and Scandinavian neurologists appear quite reluctant in this regard (0.6%) [Oger, 2007].

Also in 1991, Likosky and co-workers conducted a placebo-controlled single blinded study of 42 patients with what they termed 'chronic-progressive MS'. Again, it is unclear whether the study population was composed of patients with PRMS, SPMS, PPMS, or a mixture of all of these phenotypes. A dose of IV CYC 500 mg daily for five times was used. The investigators reported no apparent effect on clinical disease activity after 12, 18 and 24 months. Since then, there have been many open-label trials of CYC in patients with rapidly progressive and refractory MS. In summary, all of these trials stated positive and encouraging results. In 1997 Weinstock-Guttman reported an open-label study with IV CYC (500 mg/m^2 for 5 days followed by maintenance therapy) in 17 patients with fulminant MS defined as worsening of more than one a half points on the EDSS for more than 3 months [Weinstock-Guttman, 1997]. In 24 months, 69% of patients were clinically stable or had some improvement. In 1999, two other open-label trials were reported. The first, conducted by Hohol *et al.* [1999], was an open-label observational study of 95 patients with progressive MS. They reported that 80% of the patients who received monthly CYC with IV pulses of methylprednisolone (MP) had stable or improved EDSS scores at 12 months. The second trial was reported by Gobbini and co-investigators [1999] who treated five patients with rapidly deteriorating RRMS using monthly IV CYC for 6 months followed by IV CYC on alternate months. The investigators observed rapid reduction in the number of enhancing lesions which was concordant with clinical stability. In 2001, Khan and co-workers [2001] evaluated 14 patients with rapidly deteriorating RRMS, defined as more than three points increase in EDSS score in 12 months despite immunomodulating therapy and IV steroids, who were given monthly CYC. The results were very encouraging: no further relapses were reported, and stabilization of EDSS scores occurred for up to 18 months. In 2003, Perini and Gallo [2003] reported an open-label study of 24 patients with active refractory MS who received monthly IV CYC and IV MP for 1 year followed by CYC therapy in alternate months for one extra year. They noticed significant improvement in EDSS scores, relapse rate and surrogate disease makers on magnetic resonance imaging (MRI). In 2004, Zephir and co-investigators conducted a retrospective review of the charts of 490 patients with progressive MS (362 with SPMS and 128 with PPMS) who were given 12 monthly IV pulses of CYC. After 12 months, 78.6% of patients with SPMS and 73.5% of patients with PPMS showed stabilization or improvement of their EDSS scores.

In terms of comparing different regimens of CYC, La Mantia and co-workers [1998] compared three different CYC treatment paradigms in MS patients: (1) induction followed by bimonthly IV boosters for one year (17 patients), (2) bimonthly IV boosters for 1 year without induction (15 patients), and (3) monthly IV boosters for 1 year (21 patients). The percentage of stable patients was significantly higher in the first and the third groups. Myelotoxicity occurred only in the first group, and bronchopneumonia was observed in the second group. No major side effects were observed in the third group. The study suggested that monthly pulses of CYC are effective and safe in MS patients.

Based on all of these studies it is hard to conclude how effective CYC is in patients with MS. Specifically, it was never definitely determined which patient population may benefit, and which treatment paradigm should best be utilized. The potential lack of interest by a corporate entity to conduct state-of-the art clinical trials with CYC in patients with RRMS, SPMS, or PPMS has perhaps been the single biggest obstacle in determining its role as a disease-modifying agent.

Combination of cyclophosphamide and interferon beta in MS

In 2004, Patti and co-investigators reported 10 patients with rapidly worsening MS refractory to interferon beta (IFNβ) (six patients on IFNβ-1a, Avonex and four patients on IFNβ-1b, Betaseron) therapy in whom monthly IV CYC was added to the treatment regimen. Addition of CYC led to significant reduction in the

number of relapses, EDSS scores and radiological disease burden. Importantly, these findings were sustained for 36 months after cessation of CYC.

In 2005, Smith and co-workers reported the results of a trial of 59 patients with IFNβ-refractory RRMS who were treated with a combination of IV CYC and IFNβ-1b (Betaseron). This was a randomized, single-blind, parallel-group, multicenter trial in MS patients with a history of active disease during IFNβ treatment. Patients were randomized to either IV CYC 800 mg/m^2 plus IV MP 1 g or IV MP once a month for 6 months and then followed for an additional 18 months. All patients received 3 days of IV MP 1 g at screening and 30 μg IFNβ-1a IM weekly for the entire 24 months. The primary endpoint was change from baseline in the mean number of enhancing lesions on MRI. Secondary clinical endpoints included time to treatment failure. Fifty-nine patients were randomized to treatment: 30 to CYC/MP and 29 to MP. Change from baseline in the number of enhancing lesions was significantly different between treatment groups at 3, 6 and 12 months with fewer lesions in the CYC/MP group. The cumulative rate of treatment failure was significantly lower in the CYC/MP group compared with the MP group. The CYC/MP combination was also well tolerated.

Cyclophosphamide versus mitoxantrone in MS

There is only one trial that has compared CYC with mitoxantrone, another alkylating chemotherapeutic agent that was approved for patients with PRMS and SPMS in 2002 [Hartung *et al.* 2002]. This open-label study that compared the efficacy and safety of CYC and mitoxantrone was conducted by Zipoli and co-workers in 2007. Mitoxantrone was administered at a dose of 8 mg/m^2 monthly for 3 months and then every 3 months (total dose 120 mg/m^2), while CYC was given at a dose of 700 mg/m^2 monthly for 12 months and then bimonthly for 24 months. Of the 153 patients enrolled, 75 received mitoxantrone and 78 received CYC. After 12 months, radiological activity was reduced by 69% in mitoxantrone group and 63% in CYC group with no significant difference. Discontinuation due to side effects was more common in patients receiving CYC though the difference was not clinically significant. The overall tolerability for both agents was acceptable.

High and myeloablative (immunoablative) doses of cyclophosphamide in MS

The recognition of aberrant immune responses in patients with MS has led many experts to believe that a resetting of the immune system may lead to sustained clinical and paraclinical benefits. Pharmacological myeloablation is one strategy that would potentially allow for a re-education of the immune system. The use of myeloablative doses of CYC in patients with neurological autoimmune disorders was pioneered by Drachman and co-workers [2003], who first used this strategy to treat patients with refractory myasthenia gravis (MG). The investigators treated three patients with ablative doses of CYC (50 mg/kg/day for 4 days). All study subjects tolerated the treatment well and showed remarkable clinical improvement. In 2008, Drachman showed data on 12 patients with refractory MG treated with myeloablatives doses of CYC (200 mg/kg). Of these 12 patients, 11 displayed a dramatic clinical improvement. The high dose was not only found to be effective, but was also safe over a period of 1–9 years of follow up.

In 2005, a report of a single patient by de Bittencourt with RRMS who accidentally received a dose of 3800 mg of CYC showed no disease activity for 7 years [de Bittencourt and Gomes-da-Silva, 2005]. In 2006, Gladstone and co-workers conducted an open-label trial with 15 patients with moderate-to-severe refractory MS who were treated with CYC 200 mg/kg over 4 days. In 15 months, the investigators observed significant improvement in the quality of life and stability of the disease. In 2008, Krishnan and co-workers reported a 2-year open-label trial of nine patients with aggressive MS who were treated with CYC 50 mg/kg/day for 4 days (total 200 mg/kg) [Krishnan *et al.* 2008]. To promote immune reconstitution, CYC therapy was followed by 5 μg/kg/day of granulocyte colony stimulating factor (GCSF) until absolute neutrophil count (ANC) $>1.0 \times 10^9$ cells/L for 2 days. The investigators demonstrated a significant reduction of EDSS scores (39.4%) after a mean follow up of 12 months and a reduction in enhancing lesions by 81.4%.

The most recent publication of the use in high dose CYC (HDC) was in February 2009 by Schwartzman *et al.* These investigators treated 23 MS patients (nine patients with RRMS, 11 patients with SPMS and four patients with PPMS) with HDC. After 3.5 years of follow-up

they observed that the RRMS group benefited the most from HDC (four patients showed no progression, three patients maintained a normal neurological exam and seven patients had a significant reduction in the flare frequency). According to this trial HDC was found ineffective in SPMS and failed in two out of four PPMS patients. HDC was found safe and tolerable and mild side effects.

A therapeutic approach that achieves depletion of immune cells very similar to myeloablation (immunoablation) is the administration of the humanized recombinant monoclonal antibody alemtuzumab (Campath), which is approved for the treatment of B cell lymphoma and chronic lymphoid leukemia [Cree, 2006]. Alemtuzumab targets CD52+ T and B lymphocytes, monocytes and macrophages and leads to cell death by cytolysis and apoptosis [Flynn and Bird, 2000; Crowe *et al*. 1992]. In patients with MS, alemtuzumab was recently shown to be effective in controlling the disease and improving disability in RRMS [CAMMS223 Trial Investigators, 2008; Coles *et al*. 2006. In SPMS patients, however, a substantial decrease of Gd-enhancing lesions was followed by long-term increased brain and spinal cord atrophy and increased disability [Roccatagliata *et al*. 2007]. Thus, the optimal time for alemtuzumab therapy may be in patients with very early aggressive disease.

Myeloablation by autologous hematopoietic stem cell transplantation (HSCT) has also more recently been explored as a treatment strategy in MS patients with an aggressive disease course. There is compelling evidence that HSCT can lead to significant and sustained control of inflammatory activity in patients with MS [Freedman *et al*. 2007; Saccardi *et al*. 2006; Fassas *et al*. 2002].

Brain atrophy in MS patients treated with high-dose cyclophosphamide

Conventional neuroimaging modalities have been disappointing when correlating clinical outcomes with surrogate disease markers on MRI. Measurement of cerebral atrophy is currently being explored as a tool to monitor disease activity and the effect of pharmacotherapies in MS. Brain volume loss is now well described after immunomodulation secondary to the resolution of brain edema (pseudoatrophy) [Zivadinov *et al*. 2008]. However, there is accumulating evidence that high-dose CYC is associated with true brain atrophy. Most notably, a relatively high dose (4–8 g/m^2) that was used in the setting of autologous hematopoietic stem cell transplantation (AHSCT) was shown to be associated with long-term significant loss of brain volume unrelated to the edema resolution [Roccatagliata *et al*. 2007; Chet *et al*. 2006; Inglese *et al*. 2004]. CYC is commonly utilized in AHSCT to mobilize peripheral blood progenitor cells.

In 2004, Inglese and his co-workers studied 10 patients with rapidly evolving SPMS treated with a AHSCT regimen that incorporated CYC (4 g/m^2), there was an average annual decrease in brain volume of about 1.9% over a period of 24 months, despite only five enhancing lesions seen on triple dose follow up scans of two patients. In 2006, Chen and co-workers measured brain atrophy in nine patients undergoing AHSCT for MS. Stem cell mobilization was achieved by using IV CYC (4.5 g/m^2). From baseline to 1 month after treatment, cerebral atrophy accelerated 10-fold compared to the pretreatment period. Roccatagliata and co-workers conducted a long-term follow-up study of nine patients with MS treated with AHSCT for a mean of 63 months and observed a marked decrease of brain atrophy after the second year following treatment.

These observations suggest that brain atrophy after immunotherapy with CYC may not be due entirely to the resolution of edema, but may be related to chemotoxicity [Chet *et al*. 2006]. The most important question is which cell type in the CNS constitutes the target of CYC therapy. As mentioned above, CYC has good bioavailability in this compartment. As it eliminates predominantly rapidly dividing cells, it is conceivable that immune cells that have become intrinsic to the brain and spinal cord of patients with advanced MS are slowly being eliminated. However, it is also possible that neurons and glial cells may be adversely affected. Alemtuzumab, as mentioned above, has caused significant brain and spinal cord atrophy, a finding that raises the question whether the brain atrophy is medication-specific or simply related to intensive immunoablation. Controlled clinical trials will be required to clarify the long-term effects of CYC on brain atrophy and the potential differences between different dose regimens.

Conclusions

In the past three decades, extensive experience with CYC in patients with MS has

been accumulated. Overall, CYC is a relatively well-tolerated and promising option for patients with aggressive forms of MS.

While some clinical studies have provided conflicting results with regard to the efficacy of CYC in patients with MS, the majority of trials have shown a positive response to CYC in treatment-refractory progressive phenotypes of MS with acceptable tolerability. Differences in outcomes may be explained by different inclusion criteria and outcomes employed by different investigators.

It appears that younger patients with a shorter disease course appear to derive the greatest benefits in terms of efficacy and tolerability. Furthermore, patients with RRMS, rapidly progressing MS and PPMS or SPMS with active disease with no major medical co-morbidities seem to be the good candidates for CYC therapy. It is also conceivable that CYC could benefit patients with other inflammatory disorders of the CNS, including NMO [Hemmer and Stuve, 2007]. Poor candidates, from a safety perspective, include: pregnant and nursing women, women of child-bearing age, the elderly (due to lack of data) and patients with medical contraindication to CYC.

The most widely utilized regimen is monthly pulses of 700–800 mg/m^2 for 1 year, followed by bimonthly pulses in treatment responders. Combination therapy with IFNβ appears to be effective and safe. Refer to Table 1 for dose adjustment based on white blood cell count for the pulse regimen [Oger, 2007]. Other modified regimens that have been used are summarized in Box 1 [Smith, 2005]. Possible safety monitoring is summarized in Box 2 [Gauthier and Weiner, 2005].

An intriguing and relatively novel approach that needs to be studied more extensively is the use of myeloablative (immunoablative) doses of CYC (200 mg/kg). The efficacy and safety of this approach needs to be verified in controlled randomized trials. If successful, these trials could establish CYC as an alternative to other aggressive treatment approaches, including alemtuzumab and HSCT. Interestingly, the kinetic of immune-reconstitution with all these strategies appears to be very similar. These trials should verify effects of CYC on brain atrophy and the effect on intrathecal immune processes. It has the advantage of providing long-lasting effects with acceptable tolerability. Given the potentially serious side effects of CYC therapy and the availability of safer treatment options, the identification of an ideal patient population will remain challenging. Some of the identified side effects, including sterility and bladder cancer, will almost certainly limit the broader use of this agent.

Table 1. Dosing of cyclophosphamide based on white blood cell (WBC) count in peripheral blood (modified from Oger, 2007).

First dose of CYC	
WBC/mm^3	CYC
>4000	full dose
3000–4000	75%
2000–3000	50%
<2000	skip
Next monthly dose of CYC	
WBC/mm^3 at nadir	CYC
1500–2000	full dose
<1500	decrease by 100–200 mg/m^2
>2000	Increase by 100–200 mg/m^2

Box 1. Different dosing regimens of cyclophosphamide (modified from Smith, 2005).

1. IV induction therapy with CYC/MP: 600 mg/m^2 IV CYC given on days 1, 2, 4, 6, 8 plus IV MP given daily for 8 days.
2. IV pulse therapy with CYC/MP after MP induction (1 g daily for 5 days): IV CYC pulses begun at 800 mg/m^2 with dose escalation designed to produce leukopenia of 2000/mm^3; every 4 weeks for 12 weeks, every 6 weeks for 12, every 2 months for 12 months; 1 g IV MP given with CYC. Maximum dose 1600 mg/m^2
3. IV pulse therapy with CYC at a fixed dose: IV CYC pulses given at 800–1000 mg/m^2 every 4–8 weeks for 12–24 months; given with or without IV MP.
4. Combination therapy: IV pulse CYC therapy given concomitantly with IFNβ or glatiramer acetate for variable time periods.

Box 2. Safety monitoring for cyclophosphamide (modified from Gauthier and Weiner, 2005).

1. WBC count prior to each dose at mid-month (nadir).
2. Patients should be instructed to drink 3 l of fluid on the day of, and the day after, treatment.
3. Urinalysis and cytology should be conducted annually during treatment.
4. Cystoscopy: if cytology is abnormal; yearly after 3 years of treatment.
5. Maximum lifetime dose: 80–100 g.
6. Contraception before planning CYC in women during CBP and pregnancy test prior to dosing.
7. Seminal fluid analysis when accumulative dose exceeds 300 mg/kg.

Conflict of interest statement

None declared.

References

Balshov, K.E., Smith, D.R., Khoury, S.J., Hafler, D.A. and Weinrer, H.L. (1997) Increased interleukin-12 production in progressive multiple sclerosis: induction by activated CD4+ T cells via CD40 ligand. *Proc Natl Acad Sci USA* 94: 599–603.

Blake, D.A., Heller, R.H., Hsu, S.H. and Schacter, B.Z. (1976) Return of fertility in a patient with cyclophosphamide-induced azoospermia. *Johns Hopkins Med J* 139: 20–22.

Blumenfeld, Z. (2007) Gender difference: fertility preservation in young women but not in men exposed to gonadotoxic chemotherapy. *Minerva Endocrinol* 32: 23–34.

CAMMS223 Trial Investigators, Coles, A.J., Compston, D.A., Selmaj, K.W., Lake, S.L., Moran, S., Margolin, D.H. *et al.* (2008) Alemtuzumab vs. interferon beta-1a in early multiple sclerosis. *N Engl J Med* 359: 1786–1801.

Canadian Cooperative Multiple Sclerosis Study Group. (1991) The Canadian cooperative trial of cyclophosphamide and plasma exchange in progressive multiple sclerosis. *Lancet* 337: 441–446.

Chet, J.T., Collins, D.L., Atkins, H.L., Freedman, M.S., Galal, A. and Arnold, D.L. (2006) Brain atrophy after immunoablation and stem cell transplant in multiple sclerosis. *Neurology* 66: 1935–1937.

Coles, A.J., Cox, A., Le Page, E., Jones, J., Trip, S.A., Deans, J. *et al.* (2006) The window of therapeutic opportunity in multiple sclerosis: evidence from monoclonal antibody therapy. *J Neurol* 253: 98–108.

Comabella, M., Balashov, K., Hafler, D.A., Issazadeh, S., Smith, D., Weiner, H.L. *et al.* (1998) Elevated interleukin-12 in progressive multiple sclerosis correlates with disease activity and is normalized by pulse cyclophosphamide therapy. *J Clin Invest* 102: 671–678.

Compendium of Pharmaceuticals and Specialties. (2006) ®Cytoxan: Canadian Pharmacists Association (Canada).

Cree, B. (2006) Emerging monoclonal antibody therapies for multiple sclerosis. *Neurologist* 12: 171–178.

Cross, A.H., Stark, J.L., Lauber, J., Ramsbottom, M.J. and Lyons, J.A. (2006) Rituximab reduces B cells and T cells in cerebrospinal fluid of multiple sclerosis patients. *J Neuroimmunol* 180: 63–70.

Crowe, J.S., Hall, V.S., Smith, M.A., Cooper, H.J. and Tite, J.P. (1992) Humanized monoclonal antibody CAMPATH-1H: myeloma cell expression genomic constructs nucleotide sequence of cDNA constructs and comparison of effector mechanisms of myeloma and Chinese hamster ovary cell-derived material. *Clin Exp Immunol* 87: 105–110.

de Bittencourt, P.R. and Gomes-da-Silva, M.M. (2005) Multiple sclerosis: long-term remission after a high dose of cyclophosphamide. *Acta Neurol Scand* 111: 195–198.

De Ridder, D., van Poppel, H., Demonthy, L., D'hooghe, B., Gonsette, R., Carton, H. *et al.* (1998) Bladder cancer in patients with multiple sclerosis treated with cyclophosphamide. *J Urol* 159: 1881–1884.

Drachman, D.B., Jones, R.J. and Brodsky, R.A. (2003) Treatment of refractory myasthenia: 'rebooting' with high-dose cyclophosphamide. *Ann Neurol* 53: 29–34.

Drachman, D.B., Adams, R.N., Hu, R., Jones, R.J. and Brodsky, R.A. (2008) Rebooting the immune system with high-dose cyclophosphamide for treatment of refractory myasthenia gravis. *Ann N Y Acad Sci* 1132: 305–314.

Fassas, A., Passweg, J.R., Anagnostopoulos, A., Kazis, A., Kozak, T., Havrdova, E. *et al.* (2002) Hematopoietic stem cell transplantation for multiple sclerosis: a retrospective multicenter study. *J Neurol* 249: 1088–1097.

Flynn, J.M. and Byrd, J.C. (2000) Campath-1H monoclonal antibody therapy. *Curr Opin Oncol* 12: 574–581.

Freedman, M.S., Atkins, H.L., Arnold, D.L. and Bar-Or, A. on behalf of the Canadian BMT group (2007) Immune ablation and autologous stem cell transplantation for aggressive multiple sclerosis: interim 5-year report. *Mult Scler* 13(Suppl 2): 22(abstr).

Gauthier, S.A. and Weiner, H.L. (2005) Cyclophosphamide therapy for MS. *Int MS J.* 12: 52–58.

Gladstone, D.E., Golightly, M.G. and Brannagan, III T.H. (2007) High dose cyclophosphamide preferentially targets naïve T (CD45/CD4/RA+) cells in CIDP and MS patients. *J Neuroimmunol* 190: 121–126.

Gladstone, D.E., Zamkoff, K.W., Krupp, L., Peyster, R., Sibony, P., Christodoulou, C. *et al.* (2006) High-dose cyclophosphamide for moderate to severe refractory multiple sclerosis. *Arch Neurol* 63: 1388–1393.

Gobbini, M.I., Smith, M.E., Richert, N.D., Frank, J.A. and McFarland, H.F. (1999) Effect of open label pulse cyclophosphamide therapy on MRI measures of disease activity in five patients with refractory relapsing-remitting multiple sclerosis. *J Neuroimmunol* 99: 142–149.

Hartung, H.P., Gonsette, R., Konig, N., Kwiecinski, H., Guseo, A., Morrissey, S.P. *et al.* (2002) Mitoxantrone in progressive multiple sclerosis: a placebo-controlled, double-blind, randomised, multicentre trial. *Lancet* 360: 2018–2025.

Hauser, S.L., Dawson, D.M., Lehrich, J.R., Beal, M.F., Kevy, S.V., Propper, R.D. *et al.* (1983) Intensive immunesuppression in progressive multiple sclerosis.

A randomized, three-arm study of high-dose intravenous cyclophosphamide, plasma exchange, and ACTH. *N Engl J Med* 308: 173–180.

Hemmer, B. and Stuve, O. (2007) Revised criteria for neuromyelitis optica – a new diagnostic standard? *Nat Clin Pract Neurol* 3: 132–133.

Hohol, M.J., Olek, M.J., Orav, E.J., Stazzone, L., Hafler, D.A., Khoury, S.J. *et al.* (1999) Treatment of progressive multiple sclerosis with pulse cyclophosphamide/methylprednisolone: response to therapy is linked to the duration of progressive disease. *Mult Scler* 5: 403–409.

Hommes, O.R., Prick, J.J. and Lamers, K.J. (1975) Treatment of chronic progressive form of multiple sclerosis with a combination of cyclophosphamide and prednisone. *Clin Neurol Neurosurg* 78: 59–72.

Inglese, M., Mancardi, G.L., Pagani, E., Rocca, M.A., Murialdo, A., Saccardi, R. *et al.* (2004) Brain tissue loss occurs after suppression of enhancement in patients with multiple sclerosis treated with autologous haematopoietic stem cell transplantation. *J Neurol Neurosurg Psychiatry* 75: 643–644.

Khan, O.A., Zavartau-Hind, M., Caon, C., Din, M.U., Cochran, M., Lisak, D. *et al.* (2001) Effect of monthly intravenous cyclophosphamide in rapidly deteriorating multiple sclerosis patients resistant to conventional therapy. *Mult Scler* 7: 185–188.

Kanter, I.C., Huttner, H.B., Staykov, D., Biermann, T., Struffert, T., Kerling, F. *et al.* (2008) Cyclophosphamide for anti-GAD antibody-positive refractory status epilepticus. *Epilepsia* 49: 914–920.

Karni, A., Balashov, K., Hancock, W.W., Bharanidharan, P., Abraham, M., Kohoury, S.J. *et al.* (2004) Cyclophosphamide modulates CD4+ T cells into a T helper type 2 phenotype and reverses increased IFN-γ production of CD8+ T cells in secondary progressive multiple sclerosis. *J Neuroimmunol* 146: 189–198.

Kim, Y.H., Choi, B.K., Oh, H.S., Kang, W.J., Mittler, R.S. and Kwon, B.S. (2009) Mechanisms involved in synergistic anti-cancer effects of anti 4-1-BB and cyclophosphamide therapy. *Mol Cancer Ther* 8: 469–478.

Kovarsky, J. (1983) Clinical pharmacology and toxicology of Cyclophosphamide: emphasis on use in rheumatic diseases. *Semin Arthritis Rheum* 12: 359–372.

Krishnan, C., Kaplin, A.I., Brodsky, R.A., Drachman, D.B., Jones, R.J., Pham, D.L. *et al.* (2008) Reduction of disease activity and disability with highdose cyclophosphamide in patients with aggressive multiple sclerosis. *Arch Neurol* 65: 1044–1051.

Kurtzke, J.F. (1983) Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). *Neurology* 33: 1444–1452.

La Mantia, L., Eoli, M., Salmaggi, A., Torri, V. and Milanese, C. (1998) Cyclophosphamide in chronic progressive multiple sclerosis: a comparative study. *Ital J Neurol Sci* 19: 32–36.

Lebrun, C., Debouverie, M., Vermersch, P., Clavelou, P., Rumbach, L., de Seze, J. *et al.* (2008) Cancer risk and impact of disease-modifying treatments in patients with multiple sclerosis. *Mult Scler* 14: 399–405.

Likosky, W.H., Fireman, B., Elmore, R., Eno, G., Gale, K., Goode, G.B. *et al.* (1991) Intense immunosuppression in chronic progressive multiple sclerosis: the Kaiser study. *J Neurol Neurosurg Psychiatry* 54: 1055–1060.

Lublin, F.D. and Reingold, S.C. (1996) Defining the clinical course of multiple sclerosis: results of an international survey. National Multiple Sclerosis Society (USA) Advisory Committee on Clinical Trials of New Agents in Multiple Sclerosis. *Neurology* 46: 907–911.

Makhani, M.P., Gorman, H.M., Branson, L., Stazzone, B.L., Banwell, T. and Chitnis. (2009) Cyclophosphamide therapy in pediatric multiple sclerosis. *Neurology* 72: 2076–2082.

Masala, A., Faedda, R., Alagna, S., Satta, A., Chiarelli, G., Rovasio, P.P. *et al.* (1997) Use of testosterone to prevent cyclophosphamide-induced azoospermia. *Ann Intern Med* 126: 292–295.

Meirow, D., Epstein, M., Lewis, H., Nugent, D. and Gosden, R.G. (2001) Administration of cyclophosphamide at different stages of follicular maturation in mice: effects on reproductive performance and fetal malformations. *Hum Reprod* 16: 632–637.

Moore, M.J. (1991) Clinical pharmacokinetics of cyclophosphamide. *Clin Pharmacokinet* 20: 194–208.

Oger, J. (2007) Immunosuppression: promises and failures. *J Neurol Sci* 259: 74–78.

ONS Clinical Practice Committee. (1999) *Cancer Chemotherapy Guidelines and Recommendations for Practice*. Pittsburgh, PA: ONS, pp. 32–41.

Patti, F., Reggio, E., Palermo, F., Fiorilla, T., Politi, G., Nicoletti, A. *et al.* (2004) Stabilization of rapidly worsening multiple sclerosis for 36 months in patients treated with interferon beta plus cyclophosphamide followed by interferon beta. *J Neurol* 251: 1502–1506.

Perini, P., Calabrese, M., Rinaldi, L. and Gallo, P. (2007) The safety profile of cyclophosphamide in multiple sclerosis therapy. *Expert Opin Drug Saf* 6: 183–190.

Perini, P. and Gallo, P. (2003) Cyclophosphamide is effective in rapidly deteriorating secondary progressive multiple sclerosis. *J Neurol* 250: 834–838.

Polman, C.H., Reingold, S.C., Edan, G., Filippi, M., Hartung, H.P., Kappos, L. *et al.* (2005) Diagnostic criteria for multiple sclerosis: 2005 revisions to the 'McDonald Criteria'. *Ann Neurol* 58: 840–846.

Portaccio, E., Siracusa, G., Piacentini, S., Sorbi, S. and Amato, M.P. (2003) Safety and tolerability of cyclophosphamide 'pulses' in multiple sclerosis: a

prospective study in a clinical cohort. *Mult Scler* 9: 446–450.

Poser, C.M., Party, D.W., Scheinberg, L., McDonald, W.I., Davis, F.A., Ebers, G.C. *et al.* (1983) New diagnostic criteria for multiple sclerosis: guidelines for research protocols. *Ann Neurol* 13: 227–231.

Prineas, J.W. (1979) Multiple sclerosis: presence of lymphatic capillaries and lymphoid tissue in the brain and spinal cord. *Science* 203: 1123–1125.

Prineas, J.W. and Wright, R.G. (1978) Macrophages, lymphocytes, and plasma cells in the perivascular compartment in chronic multiple sclerosis. *Lab Invest* 38: 409–421.

Radis, C.D., Kahl, L.E., Baker, G.L., Wasko, M.C., Cash, J.M., Gallatin, A. *et al.* (1995) Effects of cyclophosphamide on the development of malignancy and on long-term survival of patients with rheumatoid arthritis. A 20-year follow up study. *Arthritis Rheum* 38: 1120–1127.

Roccatagliata, L., Rocca, M.A., Valsasina, P., Bonzano, L., Sormani, M., Saccardi, R. *et al.* (2007) The long-term effect of AHSCT on MRI measures of MS evolution: a five year follow-up study. *Mult Scler* 13: 1068–1070.

Saccardi, R., Kozak, T., Bocelli-Tyndall, C., Fassas, A., Kazis, A., Havrdova, E. *et al.* (2006) Autologous stem cell transplantation for progressive multiple sclerosis: Update of the European Group for Blood and Marrow Transplantation autoimmune diseases working party database. *Mult Scler* 12: 814–823.

Schwartzman, R.J., Simpkins, N., Alexander, G.M., Reichenberger, E., Ward, K., Lindenberg, N. *et al.* (2009) High-dose cyclophosphamide in the treatment of multiple sclerosis. *CNS Neurosci Ther* 15: 118–127.

Smith, D.R. (2005) Cyclophosphamide therapy for multiple sclerosis. In: Olek, M.J. (ed.). Multiple Sclerosis Humana Press: New York, pp. 171–191.

Smith, D.R., Balashov, K.E., Hafler, D.A., Khoury, S.J. and Weiner, H.L. (1997) Immune deviation following pulse cyclophosphamide/methylprednisolone treatment of multiple sclerosis: increased interleukin-4 production and associated eosinophilia. *Ann Neurol* 42: 313–318.

Smith, D.R., Weinstock-Guttman, B., Cohen, J.A., Wei, X., Gutmann, C., Bakshi, R. *et al.* (2005) A randomized blinded trial of combination therapy with cyclophosphamide in patients with active multiple sclerosis on interferon beta. *Mult Scler* 11: 573–582.

Steinman, L. (2001) Multiple sclerosis: a two-stage disease. *Nat Immunol* 2: 762–764.

Stuve, O., Cravens, P.D., Frohman, E.M., Phillips, J.T., Remington, G.M., von Geldern, G. *et al.* (2009) Immunologic, clinical, and radiologic status 14 months after cessation of natalizumab therapy. *Neurology* 72: 396–401.

Stuve, O., Marra, C.M., Jerome, K.R., Cook, L., Cravens, P.D., Cepok, S. *et al.* (2006) Immune surveillance in multiple sclerosis patients treated with natalizumab. *Ann Neurol* 59: 743–747.

Takamoto, S., Sakura, N., Namera, A. and Yashiki, M. (2004) Monitoring of urinary acrolein concentration in patients receiving cyclophosphamide and ifosphamide. *J Chromatogr B Analyt Technol Biomed Life Sci* 806: 59–63.

Takashima, H., Smith, D.R., Fukaura, H., Khoury, S.J., Hafler, D.A. and Weiner, H.L. (1998) Pulse cyclophosphamide plus methylprednisolone induces myelin-antigen-specific IL-4 secreting T cells in multiple sclerosis patients. *Clin Immunol Immunopathol* 88: 28–34.

Talar-Williams, C., Hijazi, Y.M., Walther, M.M., Linehan, W.M., Hallahan, C.W., Lubensky, I. *et al.* (1996) Cyclophosphamide-induced cystitis and bladder cancer in patients with Wegener granulomatosis. *Ann Intern Med* 124: 477–484.

Tamir, S. and Tannenbaum, S.R. (1996) The role of nitric oxide (NO.) in the carcinogenic process. *Biochim Biophys Acta* 1288: F31–36.

Ujházy, E., Balonová, T., Durisová, M., Gajdosík, A., Jansák, J. and Molnárová, A. (1993) Teratogenicity of cyclophosphamide in New Zealand white rabbits. *Neoplasma* 40: 45–49.

Weiner, H.L., Mackin, G.A., Orav, E.J., Hafler, D.A., Dawson, D.M., LaPierre, Y. *et al.* (1993) Intermittent cyclophosphamide pulse therapy in progressive multiple sclerosis: final report of the Northeast Cooperative Multiple Sclerosis Treatment Group. *Neurology* 43: 910–918.

Weinstock-Guttman, B. (1997) Treatment of fulminant multiple sclerosis with intravenous cyclophosphamide. *The Neurologist* 3: 178–185.

Wetzels, J.F.M. (2004) Cyclophosphamide-induced gonadal toxicity: a treatment dilemma in patients with lupus nephritis. *Neth J Med* 62: 347–352.

Zephir, H., de Seze, J., Duhamel, A., Debouverie, M., Hautecoeur, P., Lebrun, C. *et al.* (2004) Treatment of progressive forms of multiple sclerosis by cyclophosphamide: a cohort study of 490 patients. *J Neurol Sci* 218: 73–77.

Zipoli, V., Portaccio, E., Hakiki, B., Siracusa, G., Sorbi, S. and Amato, M.P. (2008) Intravenous mitoxantrone and cyclophosphamide as second-line therapy in multiple sclerosis: An open-label comparative study of efficacy and safety. *J Neurol Sci* 266: 25–30.

Zivadinov, R., Reder, A.T., Fillippi, M., Minagar, A., Stüve, O., Lassmann, H. *et al.* (2008) Mechanisms of action of disease-modifying agents and brain volume changes in multiple sclerosis. *Neurology* 71: 136–144.