The implementation of immunomodulators, the first evidence-based treatment option in the mid-1990s, heralded in a new era in multiple sclerosis therapy. Now we are again on the verge of another era: the introduction of the second generation of immune therapy for multiple sclerosis (MS). This article reviews current findings in MS therapy and gives a perspective on the new drugs that will potentially lead to the second revolution in MS treatment.

Modern multiple sclerosis treatment – what is approved, what is on the horizon

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In the mid-1990s, the implementation of the immunomodulatory drugs interferon-β and glatiramer acetate offered the first evidence-based treatment option for multiple sclerosis (MS). These new drugs were beneficial in a huge number of MS patients worldwide, leading to a delay in disease-related disability. Moreover, MS was suddenly the focus not only of patients and their physicians but also of pharmaceutical companies, with a tremendous increase in interest by scientists. As a result of these efforts the first monoclonal antibody treatment has recently been implemented in MS. It will presumably be followed by several other more specific, more effective and more comfortable therapies within the next few years. Here, we will show the current MS treatment options as well as recent progress in the field, and we will introduce the potential new treatment options for future MS therapy.

Introduction

Multiple sclerosis (MS) is an inflammatory, presumably autoimmune disease mainly afflicting the white matter of the human central nervous system (CNS) [1]. Previously, it was estimated that there was a worldwide prevalence of one million MS patients. In a recent study it was estimated that in 28 European countries with a total population of 466 million people, 380,000 individuals were affected with MS [2]. When extrapolating these figures, it can be assumed that the worldwide prevalence of MS exceeds one million people. Moreover, the total annual cost for MS in Europe has been estimated at 12.5 billion Euro [2]. Considering the fact that MS is the most common neurological disease affecting young adults in Western countries and results in early disease-related disability, this is an impressive indication of the enormous impact of this disease on the health system and economy of different countries in the world [3].

Despite decades of research on MS pathogenesis, and significant achievements within recent years, the etiology of MS still remains unknown. It is generally accepted that MS is a complex autoimmune disease, but this assumption still has to be proved, and this theory has yet to be formally established [4]. Several environmental risk factors such as vitamin D deficiency as well as infectious agents (e.g. in former years measles virus and most recently Epstein–Barr virus) have been thought to have a negative influence on the disease [5]. The most broadly accepted

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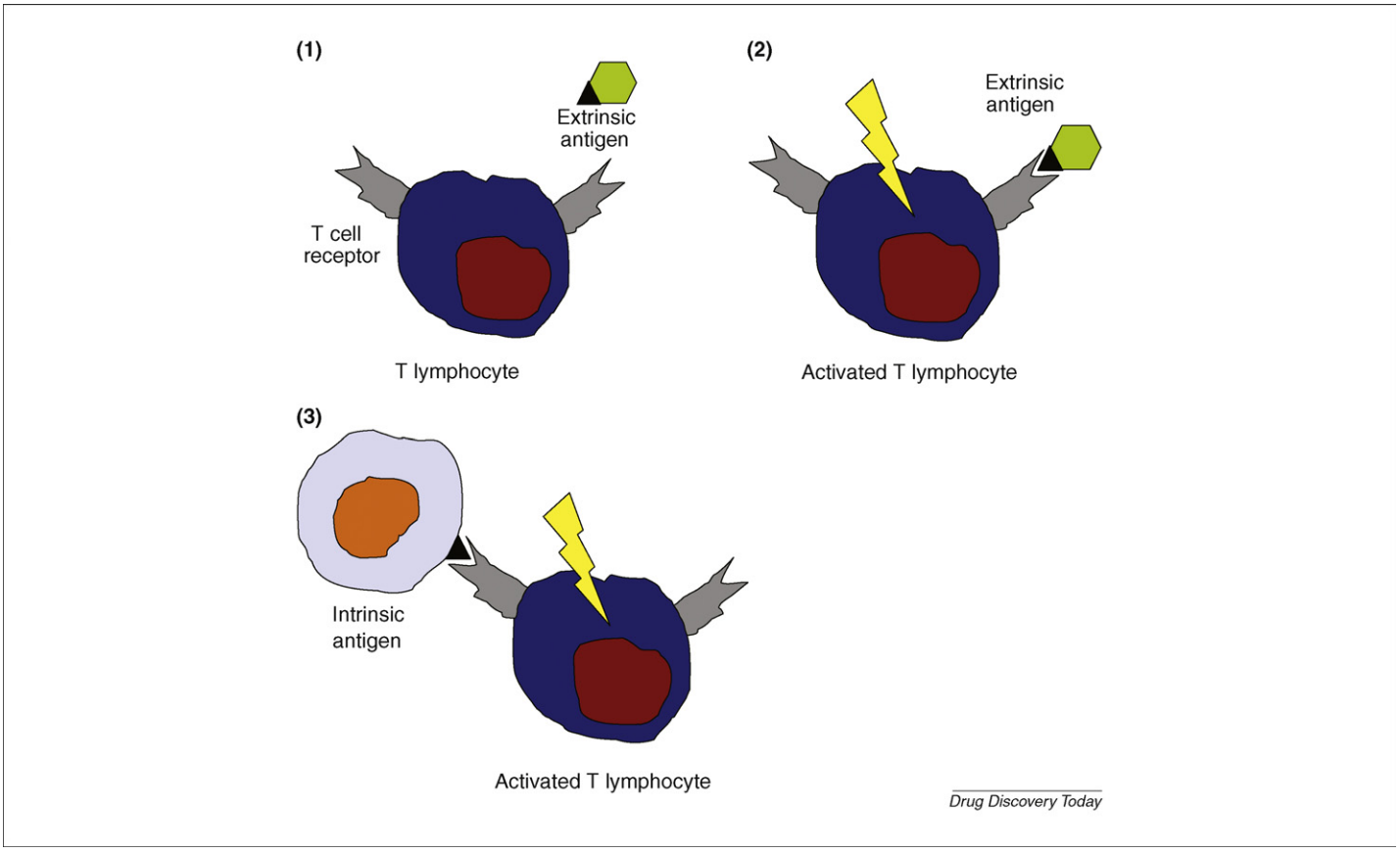

FIGURE 1

Concept of molecular mimicry in T cell-mediated autoimmune diseases: exposure to an extrinsic antigen **(1)** leads to the activation of T lymphocytes **(2)**. Later on these pre-exposed activated T lymphocytes react against intrinsic autoantigens similar to the extrinsic antigen **(3)**. These T cells have the potential to initiate a subsequent autoaggressive immune reaction. In MS, the autoantigens are suggested to be proteins of the myelin sheath within the CNS.

hypothesis on MS etiology is still the coincidence of a genetic predisposition and a certain, not yet defined, environmental factor, presumably a viral agent [6]. This environmental agent could lead to a process called 'molecular mimicry' in which a high-grade of similarity of extrinsic and intrinsic antigens causes the immune system to recognize certain autoantigens as extrinsic, and therefore becomes autoaggressive to these antigens (for details see Fig. 1) [4]. In MS, the autoantigens are thought to be proteins of the myelin sheath within the CNS.

There is growing evidence of a broad pathogenetical heterogeneity in MS. A few years ago four different histologically defined MS subtypes were described [7,8]. Despite the growing evidence of a broad pathogenetic heterogeneity, it is still suggested that T cells play a pivotal role in MS pathogenesis (for details see Fig. 2) [4].

In the early 1990s, findings on MS pathogenesis led to the development of the so-called immunomodulatory drugs interferon (IFN)-β and glatiramer acetate (GA). The term 'immunomodulatory' was used to describe a new generation of drugs for MS treatment, to distinguish them from 'immunosuppressive' therapy with drugs that had originally been developed to treat malignancies, and that have potentially severe side effects, not just on the immune system. The implementation of immunomodulatory treatment was not only new in terms of the milder effects on the immune system but also had less harmful side effects. In particular, the treatment trials of the different IFN-β preparations were innovative because they were the first ones to apply magnetic resonance imaging (MRI) to assess MS disease activity and severity [9–12]. Therefore, new gold standards not only for treatment trials but also for the treatment of MS patients in clinical routine have been established [13].

More important than the implementation of the new standards in MS is the fact that the new immunomodulatory drugs have been beneficial to hundreds of thousands of MS patients worldwide. It is not exaggerating to say that the disease has changed its face since the implementation of the new drugs leading to an improvement of the clinical symptoms and disability in a huge number of MS patients. Moreover, MS has suddenly become a great focus of interest, not only for patients and their physicians but for scientists and pharmaceutical companies as well.

As a result of these efforts, natalizumab, the first monoclonal antibody, has recently been approved for MS treatment in Europe and reapproved in North America [14]. Natalizumab will presumably be followed within the coming years by several other more specific, more effective and more comfortable therapies. Thus, we are currently on the threshold of a new generation of treatment options potentially offering further tremendous and exciting opportunities for a more individual and comfortable therapy, with great potential to provide an almost normal or at least greatly improved life for many patients suffering from this disabilitating disease which was more or less untreatable just a little more than a decade ago. It must be noted that this new generation of treatment might only be restricted to patients with relapsing MS in the near

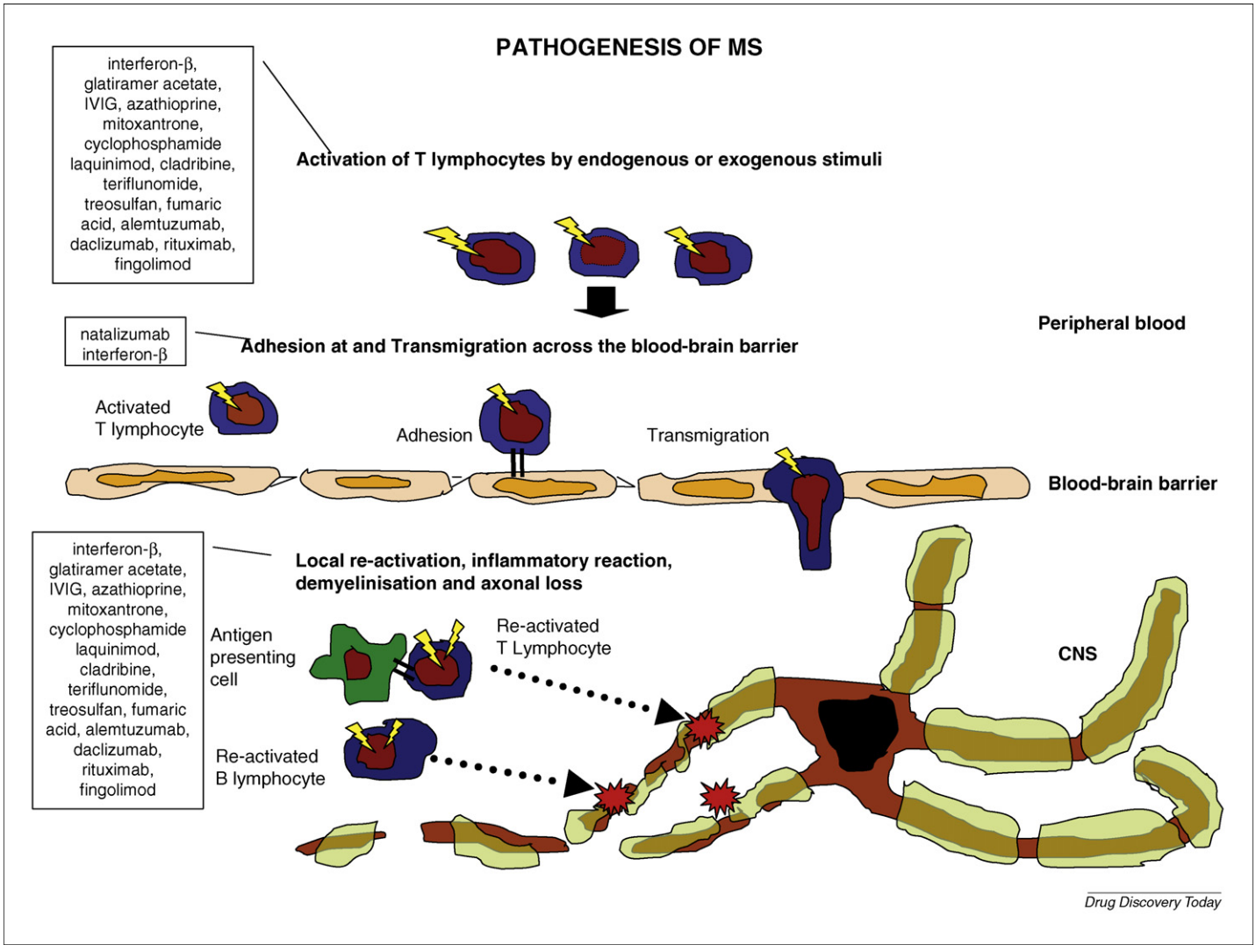

FIGURE 2

Immunological processes in the pathogenesis of multiple sclerosis. After activation in peripheral blood T lymphocytes are crossing the blood–brain barrier to transmigrate into the central nervous system (CNS). Local reactivation within the CNS leads to an inflammatory reaction resulting in demyelinization and in axonal loss. On the left side of the figure current and future MS drugs are shown according to their most probable modes of action.

future. Therefore, it will be a big challenge to establish effective therapeutic options for progressive MS in the future.

After introducing the clinical picture of MS, this article will provide an overview on current state-of-the-art treatment options in MS and also on recent scientific progress. Moreover, the most promising therapy options for future MS therapy will be introduced.

Clinical features of MS

The autoimmune process in MS leads to inflammatory lesions predominately within the white matter of the CNS [1]. These lesions can be assessed accurately by MRI scans (Fig. 3). Depending on where these lesions are located, respective clinical symptoms occur. It should be noted that usually only about one out of up to ten lesions leads to notable clinical symptoms [15]. Although theoretically any symptom resulting from a particular CNS lesion might arise, the typical syndromes at disease onset are visual disturbances, sensibility deficits and limb weakness [6]. In later stages of the disease, these symptoms are often accompanied by neuropsychological deficits (e.g. concentration disturbances, fatigue and depression), bladder and/or bowel incontinence as well as symptoms of a decreased coordination of limb or eye motion (e.g. ataxia, tremor, dysathria, double vision and vertigo). The potentially most disabling symptom, however, is loss of gait control caused by motor weakness, often in combination with coordination problems, which leads to dependency on a wheel chair in about 50% of untreated MS patients after 30 years [16,17]. Later on, even loss of arm control can occur.

In 1983 the expanded disability status scale (EDSS) was introduced by J.F. Kurtzke rating the degree of disability according to the clinical symptoms (for more information on EDSS see Table 1) [18]. In the past, several efforts have been undertaken to establish more exact scales. The EDSS, however, is still the gold standard for rating disease disability in clinical routine. In clinical research the multiple sclerosis functional composite (MSFC) has additionally been implemented as it allows observation of less severe changes of the clinical symptoms [19]. Further tools to assess disease activity and severity are the clinical relapse rate and contrast-enhancing

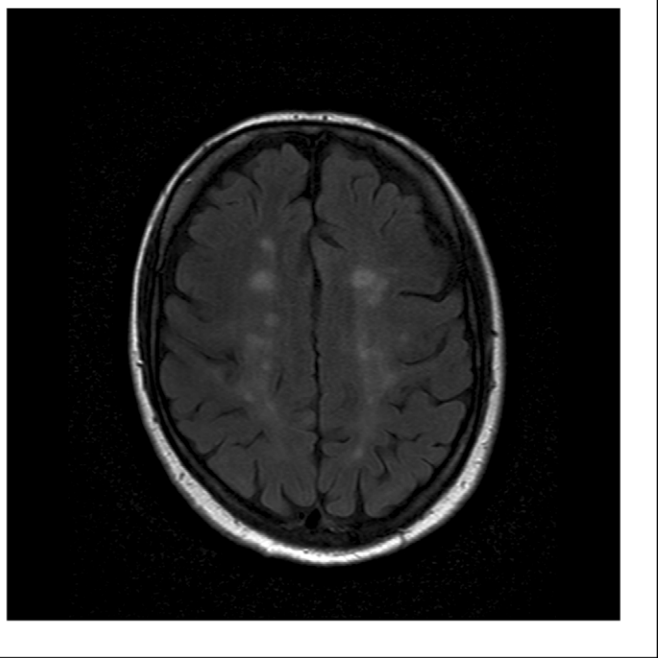

FIGURE 3

Fluid-attenuated inversion recovery (FLAIR) weighted cranial MRI scan showing multiple inflammatory white matter lesions as typically seen in multiple sclerosis.

MRI scans. Relapses are the most obvious sign of disease activity leading to a relatively acute onset of a new neurological symptom or the aggravation of a known symptom attributed to MS. Generally, relapse symptoms remain over a few weeks and then disappear either completely or incompletely. With MRI scans, both parameters for disease severity such as lesion load (e.g. cumulated number and area or volume of all MS attributed lesions) and also for disease activity (e.g. contrast-enhancing lesions and occurrence of new lesions in serial MRI) can be determined [20].

Several different disease courses have been described. The most common ones are relapsing–remitting MS (RRMS), primary progressive MS (PPMS) and secondary progressive MS (SPMS). 85% of MS patients have RRMS in the initial stage of the disease. The occurrence of acute relapses followed by a complete or incomplete remission (disappearance of relapse symptoms) is a typical characteristic at this stage. A new subtype of RRMS has only recently been introduced into clinical ratings, namely the so-called 'clinically isolated syndrome' (CIS) in which case MS is suspected [21]. CIS is diagnosed upon the first clinical relapse when the diagnostic procedure indicates that MS is highly suspected but there are still some features missing to enable a diagnosis of MS according to the most recent diagnostic criteria, the McDonald criteria (for more information on the McDonald criteria see Table 2) [8]. The introduction of the diagnosis CIS is a result of recent findings. These findings indicate that immunomodulatory treatment is more effective the earlier it is started in the course of the disease [21]. After ten years about 50% of

TABLE 1

Kurtzke expanded disability status scale (EDSS) [18]

0.0	Normal neurological examination
1.0	No disability, minimal signs in one FS
1.5	No disability, minimal signs in more than one FS
2.0	Minimal disability in one FS
2.5	Mild disability in one FS or minimal disability in two FS
3.0	Moderate disability in one FS, or mild disability in three or four FS. Fully ambulatory
3.5	Fully ambulatory but with moderate disability in one FS and more than minimal disability in several others
4.0	Fully ambulatory without aid, selfsufficient, up and about some 12 hours a day despite relatively severe disability; able to walk without aid or rest about 500 m
4.5	Fully ambulatory without aid, up and about much of the day, able to work a full day, may otherwise have some limitation of full activity or require minimal assistance; characterized by relatively severe disability; able to walk without aid or rest about 300 m
5.0	Ambulatory without aid or rest for about 200 m; disability severe enough to impair full daily activities (work a full day without special provisions)
5.5	Ambulatory without aid or rest for about 100 m; disability severe enough to preclude full daily activities
6.0	Intermittent or unilateral constant assistance (cane, crutch, brace) required to walk about 100 m with or without resting
6.5	Constant bilateral assistance (canes, crutches and braces) required to walk about 20 m without resting
7.0	Unable to walk beyond approximately 5 m even with aid, essentially restricted to wheelchair; wheels self in standard wheelchair and transfers alone; up and about in wheelchair some 12 hours a day
7.5	Unable to take more than a few steps; restricted to wheelchair; may need aid in transfer; wheels self but cannot carry on in standard wheelchair a full day; may require motorized wheelchair
8.0	Essentially restricted to bed or chair or perambulated in wheelchair, but may be out of bed itself much of the day; retains many selfcare functions; generally has effective use of arms
8.5	Essentially restricted to bed much of day; has some effective use of arms retains some selfcare functions
9.0	Confined to bed; can still communicate and eat
9.5	Totally helpless bed patient; unable to communicate effectively or eat/swallow
10.0	Death due to MS

FS: functional system, MS: multiple sclerosis.

TABLE 2

The 2005 revisions to the McDonald diagnostic criteria for multiple sclerosis [8]

Clinical presentation	**Additional data needed for MS diagnosis**
Two or more attacks[a]; objective clinical evidence of two or more lesions	None[b]
Two or more attacks[a]; objective clinical evidence of one lesion	Dissemination in space, demonstrated by: MRI[c] *or* Two or more MRI-detected lesions consistent with MS Plus positive CSF[d] *or* Await further clinical attack[a] implicating a different site
One attack[a]; objective clinical evidence of two or more lesions	Dissemination in time, demonstrated by: MRI[e] *or* Second clinical attack[a]
One attack[a]; objective clinical evidence of one lesion (monosymptomatic presentation; clinically isolated syndrome)	Dissemination in space, demonstrated by: MRI[c] *or* Two or more MRI-detected lesions consistent with MS Plus positive CSF[d] *and* Dissemination in time, demonstrated by: MRI[e] *or* Second clinical attack[a]
Insidious neurological progression suggestive of MS	One year of disease progression (retrospectively or prospectively determined) *and* Two of the following: a. Positive brain MRI (nine T2 lesions or four or more T2 lesions with positive VEP)[f] b. Positive spinal cord MRI (two focal T2 lesions) c. Positive CSF[d]

If criteria indicated are fulfilled and there is no better explanation for the clinical presentation, the diagnosis is MS; if suspicious, but the criteria are not completely met, the diagnosis is 'possible MS'; if another diagnosis arises during the evaluation that better explains the entire clinical presentation, then the diagnosis is 'not MS.' MS: multiple sclerosis; MRI: magnetic resonance imaging; CSF: cerebrospinal fluid; VEP: visual-evoked potential.

[a] An attack is defined as an episode of neurological disturbance for which causative lesions are likely to be inflammatory and demyelinating in nature. There should be subjective report (backed up by objective findings) or objective observation that the event lasts for at least 24 hours.
[b] No additional tests are required; however, if tests (MRI, CSF) are undertaken and are *negative,* extreme caution needs to be taken before making a diagnosis of MS. Alternative diagnoses must be considered. There must be no better explanation for the clinical picture and some objective evidence to support a diagnosis of MS.
[c] MRI demonstration of space dissemination must fulfill the criteria derived from Barkhof *et al.* [103] and Tintore *et al.* [104].
[d] Positive CSF determined by oligoclonal bands detected by established methods (isoelectric focusing) different from any such bands in serum, or by an increased IgG index [105–107].
[e] MRI demonstration of time dissemination must be fulfilled.
[f] Abnormal VEP of the type seen in MS [108].

untreated RRMS patients experience a slower and more silent progression of disability without any, or with only a few, acute relapses indicating the switch to the SPMS course [8,16,17].

By contrast, PPMS accounts for about 15% of MS. The typical pattern of PPMS is a slow disease progression right from the onset of the disease without any or with only a few relapses in the course of the disease. It is important to know that these different types of progression, in combination with the clinical disease activity, are the most important parameters to determine the individual MS-specific therapy. Whereas more or less all potential treatment forms can be applied in RRMS, there is still no effective treatment for PPMS.

Approved and established treatment

As mentioned above, MS is a very heterogeneous disease both in terms of pathogenesis as well as in terms of individual clinical MS-related symptoms. This must be taken into account by the physician when treating an individual MS patient. Modern MS therapy is based on three columns:

1. Treating acute relapses.
2. Disease-modifying treatment.
3. Symptomatic treatment.

Treatment of acute relapses aims to treat relapse-related symptoms rather than to have an impact on the long-term course of the disease. By contrast, disease-modifying drugs are used to influence disease activity, and therefore impending disability, over time. Disease-modifying treatment normally starts with basic therapy applying the classical immunomodulators. If basic therapy does not have favorable results, basic therapy will be switched to escalating therapy.

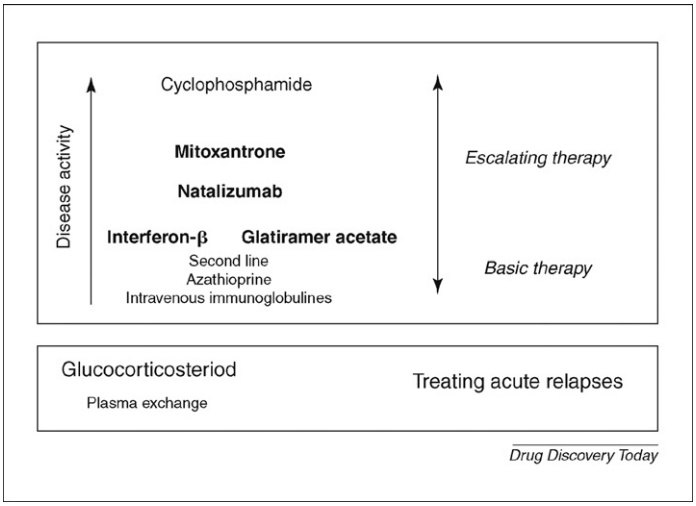

FIGURE 4

Overview on recommendations of consensus statements of a panel of German-speaking MS experts according to the best available evidence for current treatment in MS.

We have to state here that the concept of 'basic therapy' followed by 'escalating therapy' is not evidence-based in a strict sense, because no larger clinical trials have been performed comparing the basic therapies with escalating therapies. Some authors even argue in favor of an induction therapy followed by a maintenance treatment [22]. In this review we follow the recommendations of a panel of German-speaking MS experts in which they recommend treatment based on the best available evidence for their consensus statements [23,24]. An overview on these recommendations is given in Fig. 4. According to these recommendations the terms 'basic therapy' and 'escalating therapy' are applied for this report.

In contrast to relapse treatment and disease-modifying therapy, which both usually use anti-inflammatory drugs, symptomatic treatment is much more individualized and heterogeneous. Its focus is to improve the disease burden of individual patients by treating individual clinical symptoms. It is based not only on pharmacotherapy but also on physical therapy, psychological therapy and, if necessary, even on surgical procedures. The different symptomatic treatment forms are much too complex to be presented here, but Kesselring and Beer as well as Krupp and Rizvi provide excellent and comprehensive overviews on this topic [25,26]. Moreover, recommendations about symptomatic treatment of MS have also been established by the Multiple Sclerosis Therapy Consensus Group of the German Multiple Sclerosis Society [27].

Treating acute relapses

Glucocorticosteroids are still the gold standard in treating acute relapses in MS. Glucocorticosteroids have been demonstrated to shorten the duration of relapses and the severity of relapse-related symptoms. It is not clear whether glucocorticosteroids also have some long-term effects on disease activity [28,29]. A recent study examined the effect of intravenous methylprednisolone followed by oral prednisolone tapers for the treatment of relapses in neurologic disability in patients with RRMS compared with intravenous methylprednisolone only, one year after the treatment of a relapse. It demonstrates no benefit in neurologic outcome after one year for intravenous methylprednisolone followed by oral prednisolone tapers [30].

In some patients the clinical response to steroid treatment is not effective. Subsequent *plasma exchange* can be an alternative for patients suffering from severe steroid-resistant relapses or specific MS variants. One uncontrolled open label trial, one placebo-controlled trial and some case series all showed that nearly three quarters of patients treated had a good or very good outcome after plasma exchange following unsuccessful steroid treatment [31,32]. In summary, these studies demonstrate the benefit of subsequent plasma exchange in certain subgroups of MS patients. These findings also support the hypothesis that autoantibodies and complement factors play an important part in the pathogenesis of the disease.

Basic therapy

A new era in MS therapy started in the 1990s when the new drugs IFN-β-1b (in North America: Betaseron™, in Europe: Betaferon™, Bayer) and IFN-β-1a (Avonex™, Biogen Idec; Rebif™, MerckSerono) proved to be effective. The role of IFN-β and GA in RRMS is without controversy [33,34]. In recent years efforts have been undertaken to broaden the indications for these immunomodulators in early stages and in progressive forms of the disease.

Concerning the efficacy of this polypeptide-based therapy, recently one retrospective and one prospective open-label study have been published, comparing the effect of IFN-β preparations with GA in RRMS patients [35,36]. The data showed no significant difference in the primary outcome parameters.

In summary, it can be stated that all available IFN-β preparations and GA provide comparable treatment. They improve the clinical course of the disease by reducing the relapse rate by approximately 30%, by ameliorating relapse severity and by leading to a delay of the progression of disability in RRMS patients. Furthermore, basic therapy has been shown to result in a reduction of MRI parameters for disease activity and disease severity. The main differences are the application form (subcutaneously versus intramuscularly) and the application frequency (daily versus up to once weekly). It is also important to note that the long-term effects of immunomodulatory drugs are currently a matter of ongoing discussion, as evidence is limited for the delay of long-term disability as achieved by the currently available drugs. An important problem is that it is not possible for ethical reasons to study disability in a placebo-controlled manner over a long-term period that would, in a strict sense, be mandatory in a life-long disease like MS. But nevertheless, evidence exists that demonstrates a good long-term efficacy and tolerability.

Interferon-β

Recently, IFN-β-1b was, in addition to the two available IFN-β-1a preparations, also shown to be effective for prophylactic treatment after the first attack (CIS). A multicentre double-blind, randomized trial assessed the efficacy and safety of IFN-β-1b 250 μg every second day given as subcutaneous injections in patients with CIS and MRI findings suggestive of MS. After two years, treatment reduced the risk of conversion to the primary end points including the time to clinically definite multiple sclerosis (CDMS) and also the time to the diagnosis of MS according to the McDonald criteria (Table 2) by about 50% [37].

IFN-β-1a (Avonex 30 μg) was demonstrated to be effective for the early-stage treatment several years ago. Two trials with intramuscularly injected IFN-β-1a initiated after CIS showed a delay of a conversion to CDMS [38,39].

Despite the evidence supporting early initiation of therapy, it should be mentioned that starting IFN-β early is not effective in all patients as up to one-third of IFN-β-treated MS patients can be treatment nonresponders [37,40].

Another problem with early treatment onset is the so-called 'benign MS' which should be taken into consideration by the physician at the initiation of early treatment. About 10% of MS patients have a benign course of their disease with only few relapses and no, or only few, symptoms over years. There is up to now a complete lack of objective markers to separate these patients, soon after the initial diagnosis, from MS patients with more aggressive disease courses. Patients with benign MS would be unnecessarily exposed to therapy which would probably be long-lasting and potentially harmful. Growing evidence demonstrates that the inflammatory process is most active at the beginning of disease [38]. Therefore, most experts consider early treatment,

particularly in patients with a high lesion load in MRI scans or after a severe first relapse [27]. Some ongoing prospective studies will further analyze and demonstrate the long-term effects of early treatment initiation. Another problem with IFN-β treatment is that it can cause neutralizing antibodies (NABs) to occur with potential loss of effectivity [41,42]. The prevalence of NAB was up to 42% in different studies and was found to be dependent on the different preparations, dosages and application forms [41,42].

A recent multicentre, open-label, observational study followed up patients who participated in an IFN-β-1b pivotal study for 16 years [43]. During long-term treatment IFN-β-1b showed an excellent safety and tolerability profile. Furthermore, side effects and NABs decreased with time.

Glatiramer acetate (GA)

Besides IFN-β preparations, GA 20 mg s.c. once daily is an effective alternative to treat RRMS. The oral administration of GA in RRMS was, however, ineffective: a recently published double-blind randomized multicentre study compared the effect of two different oral doses of GA (50 mg or 5 mg once daily) with placebo and no difference between the cumulative number of confirmed relapses or MRI activity was observed [44]. Another study showed that immunomodulatory treatment in slowing disease progression in patients with PPMS was ineffective. This trial evaluated the effect of GA given subcutaneously on accumulation of disability in PPMS. The study was aborted after two years because at that time there were no significant effects of GA versus placebo [45]. Subgroup analysis demonstrated that male patients with PPMS might have a potential benefit from GA treatment.

By contrast, NAB plays no role in treatment with GA. An open-label study indicates that switching to GA could be an effective alternative in IFN-β-treated patients with high-titered NAB [46]. A recent study evaluated the safety and efficacy of long-term GA therapy. Forty-six patients with RRMS were treated for up to 22 years in an ongoing, open-label study [47]. It showed a low rate of relapse and EDSS progression in RRMS patients. Furthermore, GA was safe and generally well tolerated.

Another recently published study showed that GA can be an effective alternative following escalating therapy with mitoxantrone [48].

Intravenous immunoglobulins (IVIGs)

Intravenous immunoglobulins (IVIGs) are an established therapy for demyelinating diseases of the peripheral nervous system and other autoimmune processes [49]. IVIGs have been suggested to have beneficial effects in MS.

Four double-blind IVIG studies were performed in RRMS. A meta-analysis of these four trials demonstrated that IVIG reduces the relapse rate, and presumably also the disease progression [49]. As compared to the pivotal IFN-β and GA treatment trials, the number of included patients in the study was small and each single study had its methodological weakness. The authors of the meta-analysis, however, still concluded that IVIG could be considered in patients who do not tolerate IFN-β and GA [49]. In patients with a first episode of demyelinating disease, IVIG treatment delays the time until the second relapse and thereby to the diagnosis of definite MS [49]. All these studies indicate that IVIG as an add-on therapy to methylprednisolone does not have an additional beneficial effect for patients with an acute MS relapse [50]. In secondary progressive MS, IVIG also had no significant effects on disease progression, relapse rate or new MRI lesions [51]. By contrast, IVIGs are a reasonable treatment option in pregnant MS patients with active disease as they were seen to lead to a stabilization of the disease course without any evidence for teratogenic effects [52].

In conclusion, according to the recommendations of the above-mentioned consensus paper from a panel of German-speaking MS experts, and according to conclusions as stated by the authors of a meta-analysis, the quality of data on IVIG treatment can only be seen as being limited, although some studies have shown a beneficial effect [23,24,49]. Therefore, IVIG treatment is considered as a second-line option in MS.

By contrast, IVIGs are a reasonable treatment option in pregnant MS patients with active disease [52].

Azathioprine

Azathioprine has been proposed in the treatment of MS since 1971 and continues to be used in many MS centers.

Recent publications, suggesting its efficacy in reducing MRI lesion load and in refractory IFN-β-treated MS patients, have raised new hope in this drug [53,54]. Its therapeutic benefit and potentially less harmful side effects (as compared to other immunosuppressive agents) are generally considered favorable, but concerns about a possible risk of malignancies and also teratogenicity have limited its use in the past. The occurrence of unexpected adverse events in clinical trials in recent years has aroused the interest in the safety profile of drugs. Basically, the safety profile of azathioprine is acceptable, if strategies for the management of expected adverse events are adopted, and if dosage and treatment duration are maintained, and if long-term monitoring to evaluate the risk of cancer is conducted [55]. Several studies suggest that the combination of azathioprine with IFN-β preparations is well tolerated but without a clear synergistic effect [56].

Despite new hope for this drug, azathioprine should only be applied as a second-line option for MS therapy due to the lack of controlled efficacy studies, varying results of existing studies and its potentially harmful side effects (Fig. 4).

Escalating therapy

Natalizumab

Natalizumab is the first targeted therapy in MS which inhibits an essential mechanism for lymphocyte entry into the CNS and thus prevents the occurrence of new demyelinating lesions. Natalizumab (Tysabri™, Biogen Idec) is a new disease-modifying therapy approved in 2006 in the EU and reapproved in the USA for the treatment of RRMS. Natalizumab, a recombinant, humanized antibody, targets the adhesion molecule α4β1-integrin on the surface of leukocytes and blocks its interaction with its ligand vascular cell adhesion molecule-1 (VCAM-1) which is located on activated endothelial cells of the blood–brain barrier (Fig. 2) [57].

As a result of natalizumab treatment, relapse frequency was reduced by more than two-thirds and disease progression was slowed during a two-year observation period [58]. In contrast to its impressing efficacy, rare but serious adverse effects have occurred in the clinical trials. In three patients progressive multifocal leucoencephalopathy (PML) was diagnosed, with lethal outcome in

TABLE 3

Overview on the new drugs under evaluation as presented in more detail in section 'Potential future therapies'

	Mechanism of action	Results	Future perspectives
Unspecific immunosupression			
Laquinimod	Orally bioavailable immunomodulator	Phase II trial: superior to placebo in reducing MRI lesions	Phase III trial is on the way
Cladribine	Orally bioavailable, lymphocytotoxic effects	Phase II trial: active MRI lesions were reduced. No significant clinical improvement	Phase III trial is ongoing
Teriflunomide	Orally bioavailable, reduced B and T cell proliferation	Phase II trial: MRI lesions were reduced, delayed progression of the EDSS score	Phase III trial is ongoing
Treosulfan	Orally bioavailable, inhibition of proliferation and induction of apoptosis of mononuclear cells	Phase I trial: induced a stabilization or even improvement of the disability, MRI lesions were significantly reduced	Larger trials are on the way
Fumaric acid	Orally bioavailable, shift to Th2 cytokines and also has neuroprotective properties	Phase II trial: reduced number of active MRI lesions	Phase III trial is ongoing
Specific immunosupression			
Alemtuzumab	T cell depletion by binding CD52	Phase II trial: reduced relapse rate and disability progression, significantly reduced MRI lesions	Two Phase III trials are ongoing
Daclizumab	Binding CD25, reduces T cell activation and stimulates immunoregulatory natural killer cells	Phase II trial: improvement of several clinical parameters and reduction of number of MRI lesions	Phase III trial is ongoing
Rituximab	Targets the CD20 antigen and effectively depletes B lymphocytes from cerebrospinal fluid and blood	Phase II trial: significantly reduced inflammatory MS lesions and clinical relapses	Larger trials are ongoing
Fingolimod	Orally bioavailable, inhibited lymphocyte egress from secondary lymphatic organs	Phase II trial: reduced relapse rate, reduced active MRI lesions	Phase III trial is ongoing

MS, multiple sclerosis; MRI, magnetic resonance imaging; EDSS, expanded disability status scale according to Kurtzke [18]. The modes of action, the results from finalized studies and future perspectives are demonstrated.

two of the patients and severe disability in the third. PML is a devastating disease caused by JC virus and is usually found only in patients that are severely immunocompromised, such as in late phases of AIDS or in malignancies. Of the three known cases of PML, two patients with MS had been treated with combination therapy with intramuscularly applied IFN-β-1a, and the third with Crohn's disease had received other immunosuppressive therapy. It is uncertain, however, whether JC virus reactivation was caused by general immunosuppression, or by a more specific effect related to the particular mode of action of this drug [59]. As of end of March 2008, with worldwide approximately 36,700 patients having been treated with natalizumab, no further cases of PML have been identified [60]. In contrast to this, most recently reports occurred that in the end of July 2008 two more MS patients were diagnosed with PML. Both European patients had received natalizumab monotherapy for more than 14 months, one has been hospitalized, the other one is at home and ambulatory (see link to the press release of Biogen Idec http://investor.biogenidec.com/phoenix.zhtml?c=148682&p=irol-SECText&TEXT=aHR0cDovL2NjYm4uMTBrd2l6YXJkLmNvbS94bWwvZmlsaW5nLnhtbD9yZXBvPXRlbmsmaXBhZ2U9NTgwMjYwMSZkb2M9MQ%3d%3d). It will be very important to analyze the disease history of these two patients in detail to draw further conclusions about the usage of natalizumab in clinical routine. In the Natalizumab Safety and Efficacy in RRMS (AFFIRM) trial, infusion-related hypersensitivity reactions were observed in 4% of patients, usually within two hours after starting the infusion [61]. All hypersensitivity reactions resolved without any persisting deficits for the patients.

Considering its beneficial effects and also its potentially harmful side effects, several experts have recommended that natalizumab should be used in RRMS patients as a subsequent escalating therapy after IFN and GA, or in patients without basic therapy but with a highly active disease course [24].

Mitoxantrone

Mitoxantrone is an antineoplastic agent approved for the treatment of secondary progressive and rapidly worsening RRMS. A new strategy using drugs such as mitoxantrone with global immunosuppression which were effective in preventing organ transplant rejection, showed also immunomodulatory processes that might modify disease activity in RRMS [62]. Mitoxantrone decreased not only the relapse rate compared with placebo but also prolonged significantly the time to confirm progression. It also appears to be an effective treatment to stabilize rapidly worsening, treatment-refractory MS [63].

There are also some important data in two combination studies of mitoxantrone with IFN-β preparations and GA. An open-label, add-on, pilot study was performed in ten patients with MS who still had active disease despite at least six months of IFN-β-1b therapy [64]. With the addition of mitoxantrone the mean frequency and volume of gadolinium-enhancing lesions decreased by 90% and 96%, respectively, after seven months, and the relapse rate was reduced by 64%. In another open-label study, 27 consecutive patients with clinically active RRMS had received variable dose regimens of mitoxantrone, including monthly infusions for three to six months followed by mitoxantrone every three months, in combination with GA [48]. At a mean follow-up time of 36 months (range 16–66 months) from the first dose of mitoxantrone, EDSS scores (Table 1) and relapse rate were significantly reduced compared with baseline therapy.

Unfortunately the use of mitoxantrone is limited by dose-related cardiotoxicity after about just two years of treatment in a life-long disease. In recent years, reports on treatment-related acute leukemia will probably have further impact in reducing the broad use of mitoxantrone [65]. Mitoxantrone will still be applied in patients with active SPMS or for patients in whom it is not possible to treat with natalizumab.

Cyclophosphamide

Cyclophosphamide (CFX) is a cytotoxic and immunosuppressive agent, used in systemic autoimmune diseases. It is an alkylating drug that binds to DNA and interferes with mitosis and cell replication. CFX causes suppression of cell-mediated and humoral immunity through its effects on B and T cells [66,67]. Controversial results have been reported on its efficacy in MS.

Since the early 1990s a series of studies have been performed to test the efficacy of CFX in patients with rapidly worsening or treatment-refractory MS [68]. In summary, data from several open-label studies indicate that patients with rapidly worsening, treatment-refractory, RRMS might benefit from treatment with intravenous CFX.

In two recently published trials patients with RRMS who did not respond to IFN-β, the combination of CFX and IFN-β-1a reduced clinical disease activity and gadolinium-enhancing MRI lesions in the brain [69].

In particular, after the availability of natalizumab as a specific therapy, CFX should only be applied as a second-line option for MS therapy because of its potentially harmful side effects and the lack of controlled efficacy studies.

Potential future therapies

At the present time, numerous studies are testing several new drugs for their efficacy in MS treatment (for an overview see Table 3). The following section introduces substances that have the potential to follow natalizumab as a second generation of drugs which will possibly be approved for MS therapy within the next five to ten years. These potential MS drugs of the future can be roughly classified into two groups: the first group contains new treatment options for MS that result in unspecific immunosuppression and have already been applied in the treatment of other diseases, in particular, autoimmune diseases and malignancies; drugs from the second group, mainly monoclonal antibodies, interfere with a specific mechanism that has been determined to be pathogenetically relevant in MS.

Finally, current efforts on neuroprotective therapies that will probably account for the third generation of MS drugs will be briefly presented.

Besides all potential benefits that might be achieved by these future drugs it is possible, as in the case of all new drugs, that unexpected long-term side effects might occur. The following list of potential future therapies makes no claim to be complete.

Immunosupression

There are several reasons why drugs causing a generalized immunosupression can be helpful in the treatment of MS:

- Currently, substances that can be administrated orally are available. They have been seen to be safe and to have tolerable side effects in other diseases.
- The pathogenetic mechanisms of MS are heterogenous and complex.
- Various antigen specific immunotherapies were seen not to be effective in MS.
- A specific antigen that triggers MS has not yet been found.

Laquinimod

Laquinimod, a quinoline-3-carboxamid, is an orally bioavailable immunomodulator. In EAE it is much more potent than its precursor substance, linomide [70]. In a Phase II study linomide reduced active MS-related MRI lesions effectively [71]. The subsequent Phase III study of linomide was previously interrupted by severe complications such as serositis and myocardial infarction [72]. By contrast, in two Phase II trials, including 209 and 306 patients with RRMS, laquinimod was well tolerated and did not cause any severe side effects [73,74]. Treatment over a period of 24 weeks with high doses (0.3–0.6 mg/day orally) was superior to placebo in reducing active MRI lesions. A larger Phase III trial is currently on the way.

Cladribine

Cladribine (Mylinax), belonging to the group of purine nucleoside analogs, has long-lasting lymphocytotoxic effects. Parenteral therapy with cladribine was seen to be effective in Phase II and Phase III trials [75]. According to positive results in the animal model of MS, experimental autoimmune encephalomyelitis (EAE) and a reasonable safety profile in a Phase II study of parenteral cladribine in MS, an oral tablet formulation of cladribine is currently under investigation in a Phase III trial in patients with RRMS [76].

Teriflunomide

Teriflunomide belongs to the group of the malononitriamide agents and inhibits the mitochondrial enzyme dihydro-orotate dehydrogenase, which leads to a reduced B and T cell proliferation. It is approved in the therapy of rheumatoid arthritis [77]. The results from a recently published Phase II trial investigating MS patients are very promising. Two different doses of teriflunomide (7 mg and 14 mg once a day orally) were compared to placebo over a period of 36 weeks [78]. Teriflunomide significantly reduced the number of active MRI lesions and also the overall number of lesions. The higher dose of the substance also delayed the progression of the EDSS score (Table 1) and mildly reduced the relapse rate. Generally the drug was well tolerated, upper respiratory infections and headaches were the most common adverse effects. How effective teriflunomide actually is will be shown in an ongoing Phase III trial.

Treosulfan

Treosulfan (dihydroxybusulfan) belongs to the group of alkylating agents. Treosulfan is approved in the therapy of ovarian cancer and it seems to be an attractive substance owing to its favorable side-effect profile. The mode of action is inhibition of proliferation and induction of apoptosis of mononuclear cells [79,80]. The immunosuppressive and myeloablative properties of treosulfan even seem to exceed these of cyclophosphamide and busulfan [81].

In a small Phase I trial 11 patients suffering from SPMS, who did not respond to several immunomodulatory drugs, were treated for

one year with treosulfan. All in all, the therapy was well tolerated and induced a stabilization or even improvement of the disability, measured by EDSS (Table 1) and MSFC scores. In MRI the overall number and the number of active MS lesions were significantly reduced [80]. Further larger trials will bring more evidence.

Fumaric acid

Fumaric acid is an established therapy for psoriasis. Owing to its frequent side effects fumaric acid esters have been developed, which are tolerated better. This drug induces a shift to Th2 cytokines and also has neuroprotective properties by the generation of detoxifying enzymes acting on the Nfr2–ARE pathway [82,83]. In a Phase II trial the substance was effective, so the drug is being further evaluated in two ongoing Phase III studies [84].

Specific immunomodulation

With the development of monoclonal antibodies it is possible to interfere with specific mechanisms of the immune system. The idea of this strategy is to get the maximum effectiveness with the minimal side effects. The principal problem for specific therapies in MS is that the pathogenesis is heterogenous and complex. Nevertheless, the development of natalizumab impressively indicates that monoclonal antibody treatment can be effective in MS. It is exciting to think that further types of this new generation of drugs will be introduced in MS therapy.

Alemtuzumab

Alemtuzumab is a monoclonal antibody binding the CD52 antigen expressed by T and B cells and inducing a long-term T cell depletion [85,86]. Since 2001 it is approved in the treatment of B cell chronic lymphatic leukemia. In a Phase II trial, alemtuzumab (24 mg/day or 12 mg/day five times at month 1 and three times at months 12) was compared with IFN-β-1a (44 μg subcutaneously three times weekly) in patients with RRMS [87]. Two years of alemtuzumab treatment reduced the relapse rate and the disability progression by about 75%. Furthermore, the number of active MRI lesions significantly decreased. This impressing efficacy was unfortunately accompanied by severe and frequent adverse events. Up to one-third of the patients started to produce antibodies against the thyreotropin receptor, which induced in 10% an autoimmune thyreoditis. Moreover, in a small number of MS patients (2.8 percent) idiopathic thrombocytopenic purpura occurred with lethal outcome in one case. Because of its remarkable efficacy in RRMS it was decided to move forward with this monoclonal antibody therapy in two larger Phase III trials. Efforts have been made to improve patients' and physicians' awareness to detect and treat potentially harmful side effects early.

Daclizumab

Daclizumab is a monoclonal antibody targeting the CD25 antigen, which is the alpha-chain of the interleukin-2 receptor [88,89]. It reduces T cell activation and stimulates immunoregulatory natural killer cells. The efficacy of daclizumab was investigated in MS patients with unsatisfying response to IFN-β therapy and ongoing disease activity. Daclizumab-treated patients had a remarkable reduction of active MRI lesions, and a significant improvement in several clinical outcomes. A Phase III trial has been initiated and will bring more evidence [88,89].

Rituximab

Another rather interesting treatment option is rituximab. This monoclonal antibody targets the CD20 antigen and effectively depletes B lymphocytes from cerebrospinal fluid and blood. It is in clinical use in the treatment of B cell tumors and rheumatoid arthritis [90–92]. Its mode of action suppresses antibody production during acute infections with, for the immune system, unknown pathogens, whereas plasma cells in the bone morrow are not eliminated. Therefore, rituximab has no influence on the pre-existing protective antibody repertoire [93]. A trial of patients with PPMS did not bring convincing results. One patient was reported by Stuve *et al.*, suffering from therapy-refractory RRMS who responded very well to the treatment with rituximab. In this patient, rituximab treatment induced a significant clinical improvement, reduced active MRI lesions and depleted B lymphocytes from the cerebrospinal fluid and the blood [94]. Interestingly, positive effects were also found in a small group of patients with neuromyelitis optica [95]. This seems to be a bit surprising as in the pathogenesis of neuromyelitis optica B lymphocytes and autoantibodies against aquaporin-4 play a decisive role whereas rituximab does not influence pre-existing B lymphocytes and plasma cell. A Phase II trial including 104 patients with RRMS showed that rituximab treatment significantly reduces inflammatory MS lesions and clinical relapses over a period of 48 weeks as compared to placebo [96]. Results from ongoing larger trials will bring more evidence.

Fingolimod (FTY720)

Fingolimod (FTY720) is a metabolite of myriocin, a substance derived from *Isaria sinclarii* [97]. After *in vivo* phosphorylation, it acts as a nonselective agonist of the sphingosine-1 phosphate receptor expressed by lymphocytes. This interaction prevents lymphocyte egress from secondary lymphatic organs and subsequent migration into sites of inflammation. Fingolimod does not induce a general immunosupression because prior activation of T and B lymphocytes or T cell memory function remains intact [98].

The results of a multicentre, placebo-controlled, double-blind Phase II study are promising. Over a period of six months 281 patients with active RRMS were treated with oral FTY720 with 1.25 mg/day or 5 mg/day or placebo [99]. A total of 255 patients finished the trial and a significant number of FTY720-treated patients of both dosage groups had no relapses. Furthermore, MRI parameters of disease activity were significantly reduced by FTY720 treatment. The higher dosage of 5 mg was not superior to 1.25 mg. Both dosages induced a reduction in the overall relapses rate of 52–55%. In the follow-up trial over 18 months, where all patients were treated with verum, the efficacy of the substance was confirmed. Although FTY720 was in general well tolerated, the most common adverse events like sinusitis, headaches and gastrointestinal disturbances seemed to be increased in the group with the higher dosage. The drug also interacts with the cardiovascular system, so blood pressure and heart rate have to be monitored. Two patients treated with FTY720 owing to organic transplantation developed an edema of the macula. A Phase III study is ongoing to confirm the efficacy and to further evaluate the adverse effects.

Neuroprotection, neuronal repair and antigen therapy

Another interesting way to interfere with the pathogenesis of MS is to support neuroprotection. Sodium channel blockers such as lamotrigine and phenytoin, glutamate agonists such as riluzole, cabanoid receptor agonists or erythropoietin have, up to now, only been proven effective in animal models [100]. There is, however, a lack of larger studies with MS patients. Therefore, it has to be stated for the moment that no neuroprotective drug exists that will be in clinical use in the near future. Strong efforts are currently underway in MS research to develop new drugs that can enhance neuroprotection and neuronal repair.

Antigen therapies aim to reduce autoreactive myelin protein specific T cells by the application of major histocompatibility class peptide complexes, altered peptide ligands or by DNA vaccination with myelin genes [101]. Although these strategies were efficient in EAE, most clinical trials failed. One trial, evaluating DNA vaccination with myelin basic protein construct, showed a trend toward beneficial effects on MRI [102]. At the moment too little is known about the molecular targets inducing the autoimmune response in MS to expect a decisive breakthrough with antigen-specific therapies within the near future.

Conclusion and future prospects

A reasonable and effective set of therapeutic options is currently available for the physician to treat the formerly untreatable disease MS. Currently, several new substances are being investigated in clinical trials and at least some of them will probably be available within the next few years. These new drugs will potentially not only lead to a better efficacy in MS treatment but also to more comfortable application forms (orally, lower frequency of infusions or injections). Moreover, recent efforts in basic research are aiming to find a new category of MS drugs that will enhance neuroprotection and neuronal repair, in contrast to the primary anti-inflammatory mode of action of the currently available drugs. In summary, it is not exaggerating to say that MS has already become a treatable disease and that both MS patients and their physicians need no longer despair and can look into the future with more confidence.

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References

1 Hohlfeld, R. and Wekerle, H. (2004) Autoimmune concepts of multiple sclerosis as a basis for selective immunotherapy: from pipe dreams to (therapeutic) pipelines. *Proc. Natl. Acad. Sci. U. S. A.* 101 (Suppl. 2), 14599–14606
2 Sobocki, P. *et al.* (2007) Estimation of the cost of MS in Europe: extrapolations from a multinational cost study. *Mult. Scler.* 13, 1054–1064
3 Lassmann, H. and Ransohoff, R.M. (2004) The CD4–Th1 model for multiple sclerosis: a critical [correction of crucial] re-appraisal. *Trends Immunol.* 25, 132–137
4 McFarland, H.F. and Martin, R. (2007) Multiple sclerosis: a complicated picture of autoimmunity. *Nat. Immunol.* 8, 913–919
5 Holmoy, T. and Hestvik, A.L. (2008) Multiple sclerosis: immunopathogenesis and controversies in defining the cause. *Curr. Opin. Infect. Dis.* 21, 271–278
6 Noseworthy, J.H. *et al.* (2000) Multiple sclerosis. *N. Engl. J. Med.* 343, 938–952
7 Lucchinetti, C. *et al.* (2000) Heterogeneity of multiple sclerosis lesions: implications for the pathogenesis of demyelination. *Ann. Neurol.* 47, 707–717
8 Polman, C.H. *et al.* (2005) Diagnostic criteria for multiple sclerosis: 2005 revisions to the ''McDonald Criteria''. *Ann. Neurol.* 58, 840–846
9 (1993) The IFNB Multiple Sclerosis Study Group. Interferon beta-1b is effective in relapsing–remitting multiple sclerosis. I. Clinical results of a multicenter, randomized, double-blind, placebo-controlled trial. *Neurology* 43, 655–661
10 Jacobs, L.D. *et al.* (1996) Intramuscular interferon beta-1a for disease progression in relapsing multiple sclerosis. The Multiple Sclerosis Collaborative Research Group (MSCRG). *Ann. Neurol.* 39, 285–294
11 (1998) PRISMS Study Group. Randomised double-blind placebo-controlled study of interferon beta-1a in relapsing/remitting multiple sclerosis. *Lancet* 352, 1498–1504
12 Johnson, K.P. *et al.* (1995) Copolymer 1 reduces relapse rate and improves disability in relapsing–remitting multiple sclerosis: results of a phase III multicenter, double-blind placebo-controlled trial. The Copolymer 1 Multiple Sclerosis Study Group. *Neurology* 45, 1268–1276
13 Miller, D.H. *et al.* (1996) Guidelines for the use of magnetic resonance techniques in monitoring the treatment of multiple sclerosis. US National MS Society Task Force. *Ann. Neurol.* 39, 6–16
14 Miller, D.H. *et al.* (2003) A controlled trial of natalizumab for relapsing multiple sclerosis. *N. Engl. J. Med.* 348, 15–23
15 Barkhof, F. *et al.* (1992) Relapsing–remitting multiple sclerosis: sequential enhanced MR imaging vs clinical findings in determining disease activity. *Am. J. Roentgenol.* 159, 1041–1047
16 Confavreux, C. *et al.* (2000) Relapses and progression of disability in multiple sclerosis. *N. Engl. J. Med.* 343, 1430–1438
17 Confavreux, C. *et al.* (2003) Early clinical predictors and progression of irreversible disability in multiple sclerosis: an amnesic process. *Brain* 126 (Pt 4), 770–782
18 Kurtzke, J.F. (1983) Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). *Neurology* 33, 1444–1452
19 Cutter, G.R. *et al.* (1999) Development of a multiple sclerosis functional composite as a clinical trial outcome measure. *Brain* 122 (Pt 5), 871–882
20 Weiner, H.L. *et al.* (2000) Serial magnetic resonance imaging in multiple sclerosis: correlation with attacks, disability, and disease stage. *J. Neuroimmunol.* 104, 164–173
21 Thrower, B.W. (2007) Clinically isolated syndromes: predicting and delaying multiple sclerosis. *Neurology* 68 (24 Suppl. 4), S12–S15
22 Vollmer, T. *et al.* (2008) Glatiramer acetate after induction therapy with mitoxantrone in relapsing multiple sclerosis. *Mult. Scler.* 14, 663–670
23 Rieckmann, P. *et al.* (2004) Escalating immunotherapy of multiple sclerosis – new aspects and practical application. *J. Neurol.* 251, 1329–1339
24 Rieckmann, P. (2006) Escalating immunomodulatory therapy of multiple sclerosis. Update (September 2006). *Nervenarzt* 77, 1506–1518
25 Kesselring, J. and Beer, S. (2005) Symptomatic therapy and neurorehabilitation in multiple sclerosis. *Lancet Neurol.* 4, 643–652
26 Krupp, L.B. and Rizvi, S.A. (2002) Symptomatic therapy for underrecognized manifestations of multiple sclerosis. *Neurology* 58 (8 Suppl. 4), S32–S39
27 Henze, T. *et al.* (2006) Symptomatic treatment of multiple sclerosis. Multiple Sclerosis Therapy Consensus Group (MSTCG) of the German Multiple Sclerosis Society. *Eur. Neurol.* 56, 78–105
28 Beck, R.W. *et al.* (1993) The effect of corticosteroids for acute optic neuritis on the subsequent development of multiple sclerosis. The Optic Neuritis Study Group. *N. Engl. J. Med.* 329, 1764–1769
29 Rhen, T. and Cidlowski, J.A. (2005) Antiinflammatory action of glucocorticoids – new mechanisms for old drugs. *N. Engl. J. Med.* 353, 1711–1723
30 Perumal, J.S. *et al.* (2008) Oral prednisone taper following intravenous steroids fails to improve disability or recovery from relapses in multiple sclerosis. *Eur. J. Neurol.* 15, 677–680
31 Keegan, M. *et al.* (2002) Plasma exchange for severe attacks of CNS demyelination: predictors of response. *Neurology* 58, 143–146
32 Mao-Draayer, Y. *et al.* (2002) Treatment of steroid-unresponsive tumefactive demyelinating disease with plasma exchange. *Neurology* 59, 1074–1077
33 Comi, G. *et al.* (2001) European/Canadian multicenter, double-blind, randomized, placebo-controlled study of the effects of glatiramer acetate on magnetic resonance imaging – measured disease activity and burden in patients with relapsing multiple sclerosis. European/Canadian Glatiramer Acetate Study Group. *Ann. Neurol.* 49, 290–297
34 Rio, J. *et al.* (2005) Interferon beta in relapsing–remitting multiple sclerosis. An eight years experience in a specialist multiple sclerosis centre. *J. Neurol.* 252, 795–800

35 Haas, J. *et al.* (2005) Reduced suppressive effect of CD4+CD25 high regulatory T cells on the T cell immune response against myelin oligodendrocyte glycoprotein in patients with multiple sclerosis. *Eur. J. Immunol.* 35, 3343–3352
36 Khan, O.A. *et al.* (2001) Effect of monthly intravenous cyclophosphamide in rapidly deteriorating multiple sclerosis patients resistant to conventional therapy. *Mult. Scler.* 7, 185–188
37 Kappos, L. *et al.* (2006) Treatment with interferon beta-1b delays conversion to clinically definite and McDonald MS in patients with clinically isolated syndromes. *Neurology* 67, 1242–1249
38 Comi, G. *et al.* (2001) Effect of early interferon treatment on conversion to definite multiple sclerosis: a randomised study. *Lancet* 357, 1576–1582
39 Jacobs, L.D. *et al.* (2000) Intramuscular interferon beta-1a therapy initiated during a first demyelinating event in multiple sclerosis. CHAMPS Study Group. *N. Engl. J. Med.* 343, 898–904
40 Waubant, E. *et al.* (2003) Clinical characteristics of responders to interferon therapy for relapsing MS. *Neurology* 61, 184–189
41 Hemmer, B. *et al.* (2005) Immune response to immunotherapy: the role of neutralising antibodies to interferon beta in the treatment of multiple sclerosis. *Lancet Neurol.* 4, 403–412
42 Noronha, A. (2007) Neutralizing antibodies to interferon. *Neurology* 68 (24 Suppl. 4), S16–S22
43 Ebers, G. *et al.* (2006) The interferon beta-1b 16-year long-term follow-up study: the final results. *Neurology* 66, A32
44 Filippi, M. *et al.* (2006) Effects of oral glatiramer acetate on clinical and MRI-monitored disease activity in patients with relapsing multiple sclerosis: a multicentre, double-blind, randomised, placebo-controlled study. *Lancet Neurol.* 5, 213–220
45 Wolinsky, J.S. *et al.* (2007) Glatiramer acetate in primary progressive multiple sclerosis: results of a multinational, multicenter, double-blind, placebo-controlled trial. *Ann. Neurol.* 61, 14–24
46 Vallittu, A.M. *et al.* (2005) The efficacy of glatiramer acetate in beta-interferon-intolerant MS patients. *Acta Neurol. Scand.* 112, 234–237
47 Miller, A. *et al.* (2008) Long-term (up to 22 years), open-label, compassionate-use study of glatiramer acetate in relapsing remitting multiple sclerosis. *Mult. Scler.* January 21 [Epub ahead of print]
48 Ramtahal, J. *et al.* (2006) Sequential maintenance treatment with glatiramer acetate after mitoxantrone is safe and can limit exposure to immunosuppression in very active, relapsing remitting multiple sclerosis. *J. Neurol.* 253, 1160–1164
49 Sorensen, P.S. *et al.* (2002) Intravenous immunoglobulin G for the treatment of relapsing–remitting multiple sclerosis: a meta-analysis. *Eur. J. Neurol.* 9, 557–563
50 Sorensen, P.S. *et al.* (2004) IV immunoglobulins as add-on treatment to methylprednisolone for acute relapses in MS. *Neurology* 63, 2028–2033
51 Hommes, O.R. *et al.* (2004) Intravenous immunoglobulin in secondary progressive multiple sclerosis: randomised placebo-controlled trial. *Lancet* 364, 1149–1156
52 Achiron, A. *et al.* (2004) Effect of intravenous immunoglobulin treatment on pregnancy and postpartum-related relapses in multiple sclerosis. *J. Neurol.* 251, 1133–1137
53 Casetta, I. *et al.* (2007) Azathioprine for multiple sclerosis. *Cochrane Database Syst. Rev.* 17 (4), CD003982
54 Massacesi, L. *et al.* (2005) Efficacy of azathioprine on multiple sclerosis new brain lesions evaluated using magnetic resonance imaging. *Arch. Neurol.* 62, 1843–1847
55 La Mantia, L. *et al.* (2007) Azathioprine. Safety profile in multiple sclerosis patients. *Neurol. Sci.* 28, 299–303
56 Gold, R. (2008) Combination therapies in multiple sclerosis. *J. Neurol.* 255 (Suppl. 1), 51–60
57 Tubridy, N. *et al.* (1999) The effect of anti-alpha4 integrin antibody on brain lesion activity in MS. The UK Antegren Study Group. *Neurology* 53, 466–472
58 Polman, C.H. *et al.* (2006) A randomized, placebo-controlled trial of natalizumab for relapsing multiple sclerosis. *N. Engl. J. Med.* 354, 899–910
59 Hemmer, B. *et al.* (2006) Central nervous system infections – a potential complication of systemic immunotherapy. *Curr. Opin. Neurol.* 19, 271–276
60 Bozic, C. *et al.* (2008) Natalizumab utilization and safety in patients with relapsing multiple sclerosis: updated results from TOUCH and TYGRIS. *Neurology* 70, S02.002
61 Phillips, J.T. *et al.* (2006) Infusion-related hypersensitivity reactions during natalizumab treatment. *Neurology* 67, 1717–1718
62 Hauser, S.L. *et al.* (1983) Intensive immunosuppression 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
63 Hartung, H.P. *et al.* (2002) Mitoxantrone in progressive multiple sclerosis: a placebo-controlled, double-blind, randomised, multicentre trial. *Lancet* 360, 2018–2025
64 Jeffery, D.R. *et al.* (2005) A pilot trial of combination therapy with mitoxantrone and interferon beta-1b using monthly gadolinium-enhanced magnetic resonance imaging. *Mult. Scler.* 11, 296–301
65 Neuhaus, O. *et al.* (2006) Therapeutic role of mitoxantrone in multiple sclerosis. *Pharmacol. Ther.* 109, 198–209
66 Boster, A. *et al.* (2008) Intense immunosuppression in patients with rapidly worsening multiple sclerosis: treatment guidelines for the clinician. *Lancet Neurol.* 7, 173–183
67 Kovarsky, J. (1983) Clinical pharmacology and toxicology of cyclophosphamide: emphasis on use in rheumatic diseases. *Semin. Arthritis Rheum.* 12, 359–372
68 Perini, P. and Gallo, P. (2003) Cyclophosphamide is effective in stabilizing rapidly deteriorating secondary progressive multiple sclerosis. *J. Neurol.* 250, 834–838
69 Smith, D.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
70 Yang, J.S. *et al.* (2004) Laquinimod (ABR-215062) suppresses the development of experimental autoimmune encephalomyelitis, modulates the Th1/Th2 balance and induces the Th3 cytokine TGF-beta in Lewis rats. *J. Neuroimmunol.* 156, 3–9
71 Andersen, O. *et al.* (1996) Linomide reduces the rate of active lesions in relapsing–remitting multiple sclerosis. *Neurology* 47, 895–900
72 Noseworthy, J.H. *et al.* (2000) Linomide in relapsing and secondary progressive MS. Part I. Trial design and clinical results. *Neurology* 54, 1726–1733
73 Comi, G. *et al.* (2007) The effect of two doses of laquinimod on MRI-monitored disease activity in patients with relapsing–remitting multiple sclerosis: a multi-center, randomized, double-blind, placebo-controlled study. *Neurology* 68 (Suppl. 1), A84
74 Polman, C. *et al.* (2005) Treatment with laquinimod reduces development of active MRI lesions in relapsing MS. *Neurology* 64, 987–991
75 Romine, J.S. *et al.* (1999) A double-blind, placebo-controlled, randomized trial of cladribine in relapsing–remitting multiple sclerosis. *Proc. Assoc. Am. Physicians* 111, 35–44
76 Brousil, J.A. *et al.* (2006) Cladribine: an investigational immunomodulatory agent for multiple sclerosis. *Ann. Pharmacother.* 40, 1814–1821
77 Korn, T. *et al.* (2004) Modulation of effector cell functions in experimental autoimmune encephalomyelitis by leflunomide – mechanisms independent of pyrimidine depletion. *J. Leukoc. Biol.* 76, 950–960
78 O'Connor, P.W. *et al.* (2006) A Phase II study of the safety and efficacy of teriflunomide in multiple sclerosis with relapses. *Neurology* 66, 894–900
79 Weissert, R. *et al.* (2003) Action of treosulfan in myelin–oligodendrocyte–glycoprotein-induced experimental autoimmune encephalomyelitis and human lymphocytes. *J. Neuroimmunol.* 144, 28–37
80 Wiendl, H. *et al.* (2007) Treatment of active secondary progressive multiple sclerosis with treosulfan. *J. Neurol.* 254, 884–889
81 Sjoo, F. *et al.* (2006) Myeloablative and immunosuppressive properties of treosulfan in mice. *Exp. Hematol.* 34, 115–121
82 Sozzani, S. *et al.* (2005) Linking stress, oxidation and the chemokine system. *Eur. J. Immunol.* 35, 3095–3098
83 van Muiswinkel, F.L. and Kuiperij, H.B. (2005) The Nrf2–ARE signalling pathway: promising drug target to combat oxidative stress in neurodegenerative disorders. *Curr. Drug Targets CNS Neurol. Disord.* 4, 267–281
84 Kappos, L. *et al.* (2006) Efficacy of a novel single-agent fumarate, BG00012, in patients with relapsing–remitting multiple sclerosis: a phase 2 study. *J. Neurol.* 253, 27
85 Coles, A.J. *et al.* (1999) Monoclonal antibody treatment exposes three mechanisms underlying the clinical course of multiple sclerosis. *Ann. Neurol.* 46, 296–304
86 Lundin, J. *et al.* (2002) Phase II trial of subcutaneous anti-CD52 monoclonal antibody alemtuzumab (Campath-1H) as first-line treatment for patients with B-cell chronic lymphocytic leukemia (B-CLL). *Blood* 100, 768–773
87 Coles, A.J. (2008) Alemtuzumab compared with subcutaneous high-dose IFN-beta-1a in treatment naive relapsing–remitting multiple sclerosis: primary efficacy outcomes of CAMMS223 at 3 years. CAMMS Study Group. *Neurology* 70 (Suppl.), S22.006
88 Bielekova, B. *et al.* (2004) Humanized anti-CD25 (daclizumab) inhibits disease activity in multiple sclerosis patients failing to respond to interferon beta. *Proc. Natl. Acad. Sci. U. S. A.* 101, 8705–8708
89 Rose, J.W. *et al.* (2004) Treatment of multiple sclerosis with an anti-interleukin-2 receptor monoclonal antibody. *Ann. Neurol.* 56, 864–867
90 Monson, N.L. *et al.* (2005) Effect of rituximab on the peripheral blood and cerebrospinal fluid B cells in patients with primary progressive multiple sclerosis. *Arch. Neurol.* 62, 258–264
91 Rastetter, W. *et al.* (2004) Rituximab: expanding role in therapy for lymphomas and autoimmune diseases. *Annu. Rev. Med.* 55, 477–503

92 Stuve, O. *et al.* (2005) Clinical stabilization and effective B-lymphocyte depletion in the cerebrospinal fluid and peripheral blood of a patient with fulminant relapsing–remitting multiple sclerosis. *Arch. Neurol.* 62, 1620–1623
93 Eisenberg, R. and Albert, D. (2006) B-cell targeted therapies in rheumatoid arthritis and systemic lupus erythematosus. *Nat. Clin. Pract. Rheumatol.* 2, 20–27
94 Cross, A.H. *et al.* (2006) Rituximab reduces B cells and T cells in cerebrospinal fluid of multiple sclerosis patients. *J. Neuroimmunol.* 180, 63–70
95 Cree, B.A. *et al.* (2005) An open label study of the effects of rituximab in neuromyelitis optica. *Neurology* 64, 1270–1272
96 Hauser, S.L. *et al.* (2008) B-cell depletion with rituximab in relapsing–remitting multiple sclerosis. *N. Engl. J. Med.* 358, 676–688
97 Fujita, T. *et al.* (1994) Fungal metabolites. Part 11. A potent immunosuppressive activity found in *Isaria sinclairii* metabolite. *J. Antibiot. (Tokyo)* 47, 208–215
98 Fujimoto, T. *et al.* (1999) Ibudilast, a phosphodiesterase inhibitor, ameliorates experimental autoimmune encephalomyelitis in Dark August rats. *J. Neuroimmunol.* 95, 35–42
99 Kappos, L. *et al.* (2006) Oral fingolimod (FTY720) for relapsing multiple sclerosis. *N. Engl. J. Med.* 355, 1124–1140
100 Loeb, J.A. (2007) Neuroprotection and repair by neurotrophic and gliotrophic factors in multiple sclerosis. *Neurology* 68 (22 Suppl. 3), S38–S42
101 Lobell, A. *et al.* (2003) Suppressive DNA vaccination in myelin oligodendrocyte glycoprotein peptide-induced experimental autoimmune encephalomyelitis involves a T1-biased immune response. *J. Immunol.* 170, 1806–1813
102 Bar-Or, A. *et al.* (2007) Induction of antigen-specific tolerance in multiple sclerosis after immunization with DNA encoding myelin basic protein in a randomized, placebo-controlled phase 1/2 trial. *Arch. Neurol.* 64, 1407–1415
103 Barkhof, F. *et al.* (1997) Comparison of MRI criteria at first presentation to predict conversion to clinically definite multiple sclerosis. *Brain* 120 (Pt 11), 2059–2069
104 Tintore, M. *et al.* (2000) Isolated demyelinating syndromes: comparison of different MR imaging criteria to predict conversion to clinically definite multiple sclerosis. *Am. J. Neuroradiol.* 21, 702–706
105 Andersson, M. *et al.* (1994) Cerebrospinal fluid in the diagnosis of multiple sclerosis: a consensus report. *J. Neurol. Neurosurg. Psychiatry* 57, 897–902
106 Link, H. and Tibbling, G. (1977) Principles of albumin and IgG analyses in neurological disorders. III. Evaluation of IgG synthesis within the central nervous system in multiple sclerosis. *Scand. J. Clin. Lab. Invest.* 37, 397–401
107 Freedman, M.S. *et al.* (2005) Recommended standard of cerebrospinal fluid analysis in the diagnosis of multiple sclerosis: a consensus statement. *Arch. Neurol.* 62, 865–870
108 Chiappa, K.H. (1988) Use of evoked potentials for diagnosis of multiple sclerosis. *Neurol. Clin.* 6, 861–880