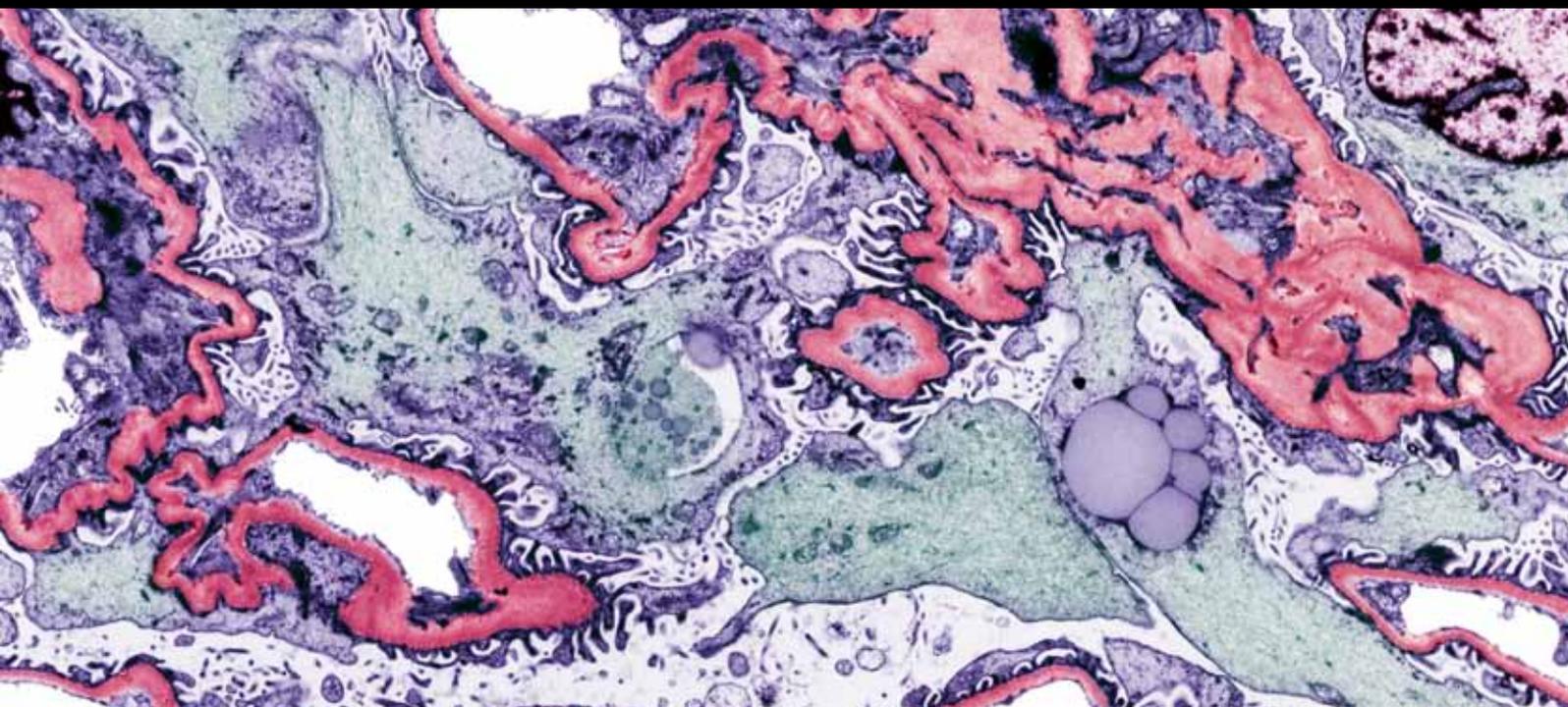


# Advances in Neuroimmunology: From Bench to Bedside

Guest Editors: Cristoforo Comi, Umberto Dianzani, Filippo Martinelli Boneschi,  
and Daniel L. Menkes





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Autoimmune Diseases

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## Editorial

# Advances in Neuroimmunology: From Bench to Bedside

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The understanding of the interactions between the immune and the nervous systems and the resultant therapeutic implications has expanded significantly in the last decade [1]. There have been significant developments in the field of neuroimmunology as new antibody-mediated disorders have been described and involvement of the immune system in the pathogenesis of neurodegenerative diseases has been established [2, 3]. These discoveries have led to novel and effective treatments, which have broadened our therapeutic options regarding neuroimmune disorders [4, 5].

The goal of this special issue was to address the translational aspects of neuroimmunology, “from bench to bedside,” in order to update clinicians on basic research discoveries that will have therapeutic clinical efficacy. Moreover, there was an emphasis on conditions that have undergone a systematic nosographic characterization which have resulted in therapeutic approaches with greater specificity. In this context, the paper entitled “*Immunotherapy of neuromyelitis optica*” provides a framework for understanding an antibody-mediated central nervous system demyelinating disease that has a different pathophysiology than multiple sclerosis (MS). This distinction is important as NMO responds to different immunomodulating agents than does MS.

The spectrum of pediatric MS has also been the focus of extensive nosographic revision in recent years, and diagnostic criteria have been recently revised by the International Pediatric Multiple Sclerosis Study Group (IPMSSG) [6].

The paper “*Pediatric multiple sclerosis: current concepts and consensus definitions*” offers a careful update on risk factors, clinical manifestations, diagnostic procedures, prognostic implications, and treatment of this increasingly frequent form of MS.

MS is a salient neuroimmunological disease for which the “bench to bedside approach” has provided the greatest therapeutic advances. Although there are more treatments for MS, the study of novel and less explored molecular pathways ought to provide relevant alternative targets. This concept is well expressed in the paper entitled “*Current understanding on the role of standard- and immuno-proteasomes in inflammatory/immunological pathways of Multiple Sclerosis*,” in which the authors describe the current knowledge on the potential role of proteasomes in MS and discuss the *pro et contra* of possible therapies for MS targeting proteasome isoforms.

Immune mediated diseases of the peripheral nervous system (PNS) are less studied than their “central” counterparts [7]. Nonetheless, important advances in both pathogenesis and treatment of inflammatory demyelinating neuropathies have been extensively evaluated in the articles authored by J. B. Weiner and P. Ripellino et al. The first publication entitled “*An update in Guillain-Barré Syndrome*” provides a comprehensive discussion of the current state of knowledge on acute inflammatory neuropathies from diagnosis to treatment. The second article, entitled “*Treatment of chronic*

*inflammatory demyelinating polyneuropathy: from molecular bases to practical considerations,*” bridges the biological rationale of immunotherapy to clinical practice also in the context of pharmacoeconomics.

Finally, the paper entitled “*An update in the use of antibodies to treat glioblastoma multiforme*” reviews the current knowledge on an expanding field immunotherapy, which is expected to have a significant impact on the progression of these high grade gliomas.

The editors believe that you will agree that this special issue will prove to be highly valuable to basic scientists and clinicians alike.

*Cristoforo Comi  
Umberto Dianzani  
Filippo Martinelli Boneschi  
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## References

- [1] P. K. Coyle, “Dissecting the immune component of neurologic disorders: a grand challenge for the 21st century,” *Frontiers in Neurology*, vol. 2, article 37, 2011.
- [2] C. Comi, M. Carecchio, A. Chiocchetti et al., “Osteopontin is increased in the cerebrospinal fluid of patients with Alzheimer’s disease and its levels correlate with cognitive decline,” *Journal of Alzheimer’s Disease*, vol. 19, no. 4, pp. 1143–1148, 2010.
- [3] G. Cappellano, M. Carecchio, T. Fleetwood et al., “Immunity and inflammation in neurodegenerative diseases,” *American Journal of Neurodegenerative Disease*, vol. 2, no. 2, pp. 89–107, 2013.
- [4] A. Haghikia, R. Hohlfeld, R. Gold, and L. Fugger, “Therapies for multiple sclerosis: translational achievements and outstanding needs,” *Trends in Molecular Medicine*, vol. 19, no. 5, pp. 309–319, 2013.
- [5] G. Cappellano, E. Orilieri, A. D. Woldetsadik et al., “Anti-cytokine autoantibodies in autoimmune diseases,” *American Journal of Clinical and Experimental Immunology*, vol. 1, no. 2, pp. 136–146, 2012.
- [6] L. B. Krupp, M. Tardieu, M. P. Amato et al., “International Pediatric Multiple Sclerosis Study Group criteria for pediatric multiple sclerosis and immune-mediated central nervous system demyelinating disorders: revisions to the 2007 definitions,” *Multiple Sclerosis Journal*, vol. 19, no. 10, pp. 1261–1267, 2013.
- [7] C. Comi, T. Fleetwood, and U. Dianzani, “The role of T cell apoptosis in nervous system autoimmunity,” *Autoimmunity Reviews*, vol. 12, no. 2, pp. 150–156, 2012.

## Review Article

# Treatment of Chronic Inflammatory Demyelinating Polyneuropathy: From Molecular Bases to Practical Considerations

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Chronic inflammatory demyelinating polyneuropathy (CIDP) is an autoimmune disease of the peripheral nervous system, in which both cellular and humoral immune responses are involved. The disease is clinically heterogeneous with some patients displaying pure motor form and others also showing a variable degree of sensory dysfunction; disease evolution may also differ from patient to patient, since monophasic, progressive, and relapsing forms are reported. Underlying such clinical variability there is probably a broad spectrum of molecular dysfunctions that are and will be the target of therapeutic strategies. In this review we first explore the biological bases of current treatments and subsequently we focus on the practical management that must also take into account pharmaco-economic issues.

## 1. Introduction

Chronic inflammatory demyelinating polyneuropathy (CIDP) is a peripheral nervous system disease that is clinically characterized by symmetrical, proximal, and distal weakness with altered sensation and hyporeflexia or areflexia [1]. Clinical course can be either relapsing remitting (RR), chronic progressive (CP), or monophasic [2]. In rare cases, CIDP displays acute onset and fast deterioration in the early phases, followed by chronic progression. This variant of CIDP, defined as “acute onset CIDP” is difficult to distinguish from Guillain-Barré syndrome (GBS) in early disease stages [3]. Epidemiological studies on CIDP report an incidence in Northern Italy around 0.6 cases per 100,000 [4]. Nevertheless, it is probable that the real incidence of CIDP is largely underestimated, due to the variety of clinical presentations and the absence of proper diagnostic markers. For this reason, a diagnosis of CIDP must be taken into consideration while examining any polyneuropathy of unknown cause.

CIDP is an autoimmune disorder, as demonstrated by a great deal of evidence [5], such as the finding of inflammation at the site of the lesion [6], response to immunomodulatory treatment [7], and possibly the presence of autoantibodies against myelin antigens [8].

Long-term prognosis of CIDP has been correlated to age at onset, response to treatment, and time from onset to the beginning of treatment: young patients with acute onset are more likely to respond to treatment than elderly ones and proximal impairment has been linked to a better prognosis than distal weakness [9, 10]. The main negative prognostic factors of CIDP are progressive course and axonal degeneration [11].

CIDP and multiple sclerosis (MS) display similarities in clinical course and pathogenesis and there are reports on cooccurrence of these two demyelinating disorders [12], but no definite conclusion whether such event was coincidental or due to common mechanisms has been reached.

Peripheral nerve injury results from a synergistic interaction of cell-mediated and humoral immune responses

directed against peripheral nerve antigens that have not been completely characterized [13].

From laboratory experiments we know that the key players in the pathogenesis of the disease appear to be T cells, especially T helper 1 (Th1) and T helper 17 (Th17) on one side and T regulatory (T reg) on the other [14]. A relevant contribution is also ascribed to the macrophagic component, cytokines, and complement activation [15–17].

CIDP is defined by a slow clinical deterioration that reaches its maximum after more than 8 weeks, differently from GBS, which is an acute and self-limiting disease. That aside, there are many similarities between these two conditions, which may even be variants of the same disease spectrum, with CIDP being the result of prolonged survival of activated T cells, not undergoing apoptosis due to a defective Fas pathway function [18–20], and GBS characterized by a self-limitation likely related to a preserved function of such apoptotic mechanism. In line with this concept, the finding that corticosteroids are effective in CIDP and not in GBS would be related to the known effect of these drugs in restoring T cell apoptosis.

Since inflammation is the core of the disease, it is not surprising that immunomodulatory treatments have a positive effect [21]. Nevertheless, it is not yet possible to predict disease progression on the basis of biological markers [22, 23] because it is likely that under the general definition of “CIDP” a broad spectrum of different forms is included [24].

In the following sections we will first discuss the biological basis for the use of immunomodulatory treatments in CIDP and subsequently illustrate our current strategy for choosing the best treatment option in everyday practice.

## 2. Biological Activity of Available Treatments

Currently available treatments for CIDP are corticosteroids, immune globulin, plasma exchange (PE), and chronic immunosuppressive agents [21, 25].

**2.1. Steroids.** Since the first report [26] of their use in CIDP in 1958, steroids have been considered a first-line therapy in CIDP. Nonetheless, their mechanism of action in patients with CIDP is not completely elucidated.

Many effects are mediated by intracellular receptors that modulate the expression of targeted genes [27]. The result of gene modulation is a pleiotropic anti-inflammatory effect mainly related to modulation of cytokines and to facilitation of apoptosis of T cells directed against the peripheral nerves [28, 29], as proved in animal models [30, 31] or in multiple sclerosis in humans [32]. During high-dose pulse therapies additional effects could occur, such as interference with intracellular signal transduction and interaction with activation of membrane-associated proteins.

A possible explanation for the variability in the clinical effect of glucocorticoids among patients and in the same patient, according to the stage of the disease, is alternative

splicing. The alpha isoform of the glucocorticoid receptor (GR $\alpha$ ) is a ligand-activated transcription factor. Alternative splicing of the glucocorticoid-receptor gene results in the expression of a GR $\beta$  isoform that exhibits negative activity [33].

A “resistant state” to steroids—that is, a reduced response to glucocorticoids or the need to increase the dose—has been described in many autoimmune conditions [34] and seems to be induced by proinflammatory cytokines [35], increased GR $\beta$  expression, or decreased glucocorticoid receptor binding. This state of glucocorticoid resistance could be positively influenced by concomitant treatment with intravenous immune globulin (IVIg) [36] with mechanisms that are still unclear but may include suppression of proinflammatory cytokines [37].

**2.2. Intravenous Immune Globulin (IVIg).** The notion that the effect of intravenously administered immune globulin (IVIg) is not limited to antibody replacement is well established. Since the first demonstrations at the beginning of the 80s [38], it has become clear that IVIg plays a role in immunomodulation and has anti-inflammatory properties. However, the anti-inflammatory activity of IVIg is still to be understood and cannot be attributed to one specific mechanism of action but rather to a variety of different ones, acting at different levels and involving both innate and adaptive immune systems [39, 40]. Specifically, anti-inflammatory activities are seen when IVIg is administered at relatively high doses compared to those used for antibody replacement. On the contrary, low doses of IVIg seem to carry out an opposite proinflammatory activity possibly through the interaction with complement and activating receptors for the crystallizable fragment portion of IgG (Fc $\gamma$ Rs) [39]. IVIg is a preparation of human polyclonal IgG obtained from plasma of several thousands of healthy donors [41], but it also contains traces of IgA and soluble molecules among which are cytokines, chemokines, soluble cytokine receptors, and receptor antagonists [39, 40]. Indeed the anti-inflammatory activity of IVIg can be related to the presence in the preparation of antibodies directed against serum proinflammatory molecules. However, IVIg seems also to act by modulating responsiveness to glucocorticoids, enhancing their anti-inflammatory effect [36].

IVIg contains antibodies with different specificities, but every antibody has the same structure: a variable portion called antigen-binding fragment (Fab) and a fixed fragment named crystallizable fragment (Fc) and both have been associated, in different ways, with anti-inflammatory activities [41, 42]. Some of the Fab-mediated activities may include neutralization of autoantibodies, cytokines, and activated complement components, anti-idiotypic activity directed against autoreactive lymphocyte clones, modulation of cell migration, targeting of specific immune cell-surface receptors, and modulation of dendritic cells function. Fc-mediated activities instead may include blockade of the neonatal Fc receptor (FcRn) and activating of Fc $\gamma$ R receptors on macrophages and other immune effector cells, upregulation of

inhibitory receptor Fc $\gamma$ RIIB, and immunomodulation by sialylated IgG [43–45].

A further role of IgG may be related to a reduction of complement uptake as they can bind to complement fragments such as C3, C4b, and C5 preventing tissue damage [46]. FcRn is found in many tissues and its activity increases the half-life of circulating IgG, as it normally binds to the Fc fragment and prevents IgG catabolism. High doses of IVIg may lead to saturation of FcRn with a consequent reduction of the half-life of autoantibodies [47]. However, this receptor displays particular affinity for deglycosylated IgG only and this aspect, which will be later discussed, tends to rule out this hypothesis [39]. Activating Fc $\gamma$ R receptors also appear to be involved, as they play a key role in the triggering of effector functions in all myeloid cells. IgG in the preparation of immune globulin may bind to activating Fc $\gamma$ Rs in the form of immune complexes thus blocking the interaction between autoantibodies and antigens. It has also been put into evidence that IgG2a and IgG2b subclasses display a greater capacity to initiate effector responses and this can be correlated to their higher affinity for activating Fc $\gamma$ Rs [39, 48].

Moreover IVIg is thought to be able to induce an upregulation of inhibitory Fc $\gamma$ RIIB receptor on effector cells whose function is to balance the activity of activating Fc $\gamma$ Rs, dismissing inflammatory response by delivering inhibitory signals [48, 49]. This theory is supported by several studies, including one conducted on patients affected by CIDP [50]. Another important aspect of IgG function is the role of glycosylation in the interaction with Fc $\gamma$ R receptors [51]. In detail, it seems that deglycosylated IgG fails to bind to such receptors [52]. Moreover, it appears that only a small percentage of glycosylated IVIg with  $\alpha$ -2,6 sialic acid linkages on Fc-linked glycans is able to exert anti-inflammatory functions [53, 54] and this could explain why high doses of IVIg are needed to observe anti-inflammatory effects [47].

It has been suggested that sialylated Fc fragments may not directly interact with Fc $\gamma$ Rs on effector cells, as they show reduced affinity, but that they may modulate inflammatory activity by binding to SIGN-RI (ICAM-3 adhesion molecule) expressed on regulatory macrophages leading to the release of soluble mediators. These mediators would then bind to effector macrophages increasing the expression of inhibitory Fc $\gamma$ RIIB which would eventually outcompete activating Fc $\gamma$ Rs, increasing the number of immune complexes needed to trigger an inflammatory response [39, 54]. Immunomodulation by glycosylation leads to further considerations on the complex environmental regulation of immune responses and weakens those hypotheses based on simple IgG-Fc $\gamma$ R and IgG-FcRn interactions. However many of these considerations have been derived from studies on animal models and must still be validated for humans. In spite of the fragmentary understanding of IVIg anti-inflammatory activity, immune globulin is successfully used in several autoimmune and inflammatory conditions including CIDP [55]. As already observed for steroids, response to treatment is often variable and this may be linked to genetic differences in immune system regulation [56] as well as glycosylation patterns in IVIg preparations.

**2.3. Plasma Exchange (PE).** There are two main techniques of standard therapeutic plasmapheresis (or plasma exchange, PE): on-line plasma separation by a cell separator (centrifuge) or by a plasma separator (i.e., membrane filtration). A standard PE protocol for neuromuscular disorders employs 4 to 5 exchanges of 1 or 1.5 plasma volumes over one week or longer (until the patient shows satisfactory improvement) [57].

The aim of this treatment is the rapid removal of circulating autoantibodies, cytokines, immune complexes, and immune cells [58] and therefore PE is used in neuroimmunological antibody-mediated diseases [59] (e.g., myasthenia gravis) to achieve fast immunosuppression. However, the duration of these effects is limited in time because of resynthesis (or even rebound production) of the respective autoantibodies and therefore PE is combined with immunosuppressive medication in chronic diseases.

PE is traditionally used in acute forms of dysimmune peripheral neuropathies such as GBS, but also patients with chronic disease such as CIDP may respond to PE in the short term, usually for 2–4 weeks [60].

Although there is now no age limit for this treatment, several possible complications, such as cardiovascular systemic reactions, electrolyte disturbances, sepsis, thrombosis and thrombophlebitis, pulmonary embolism, and subacute bacterial endocarditis, limit its chronic use in elderly patients or in patients with multiorgan disease.

**2.4. Immunosuppressive Drugs.** Azathioprine (AZA) is an antimetabolite drug that interferes with the purine pathway and therefore with DNA synthesis in cell division; it causes inhibition of proliferating lymphocytes and is often used as steroid-sparing agent.

Metotrexate is another antimetabolite interfering with the synthesis of DNA and RNA and is commonly used in autoimmune diseases (e.g., rheumatoid arthritis).

Cyclosporin A inhibits the proliferation of T cells; its action seems to be much faster than the one of azathioprine.

Mycophenolate mofetil belongs to the antimetabolite group and is a prodrug; it inhibits the proliferation of T and B lymphocytes and is generally well tolerated and relatively safe to use, although side effects include mild bone marrow suppression.

Cyclophosphamide is an alkylating agent that can be given orally or by intravenous injection to deplete T and B lymphocytes. Neutropenic infections and transient renal insufficiency as well as other mild adverse effects have been reported, but the most common adverse effects are hemorrhagic cystitis, stomatitis, leukopenia, thrombocytopenia, malignancy, and cardiomyopathy. Therefore, cyclophosphamide should only be considered for patients with a severe form of CIDP who have been refractory to other treatments.

Rituximab is a chimeric (mouse/human) monoclonal antibody directed against CD20+ B lymphocytes. It is commonly used in lymphoma and has been tried on a small series of patients with paraproteinaemic demyelinating neuropathy, with modest benefit in selected patients.

### 3. Criteria of Treatment Choice in the Era of Pharmacoeconomy

Treatment choice will depend on several variables such as initial disease severity, age, general health status, and potential contraindications [61]. The recent economic crisis is opening remarkable questions about the sustainability of expensive drugs such as IVIg in Western countries and some national audits [62] or studies [63–65] have been already performed or are still ongoing (e.g., the prospective observational study “TEPORE” in Northern Italy) to clarify this issue. The treatment with immune globulin is highly expensive, especially for chronic patients, and there are concerns about future supplies because the pool of donors is decreasing and there is the need to improve microbiological screening of plasma donors.

Subcutaneous formulation of immune globulin is now available and offers an alternative to intravenous infusions, especially for patients in working age [66].

Patients with pure motor CIDP should be treated with IVIg, since deterioration has been reported with steroids, as in MMN [67].

If a patient has only mild symptoms, a nerve biopsy could help to confirm the diagnosis and establish the need for intervention if axonal degeneration has already occurred. Mild symptomatic patients should be followed up regularly with repeated neurophysiological examinations since relapses are unpredictable and oblige to start the treatment.

Some patients will not relapse after this first course, whereas some others (with relapsing-remitting form) will need additional treatment that should be individually tailored to achieve the most cost-effective regimen.

If a patient does not respond to one of the first-line therapies, switching to another is advisable.

PE or a combination of steroids and IVIg can be started if neither of these treatments proves effective. Refractory cases may need intensive immunosuppression, according to the general principle in medicine of escalating treatment for severe disease [57].

Long-term maintenance therapy will require careful attention because of side effects of treatments on the one hand and because of the risk of relapse and axonal loss on the other. Randomized clinical trials (RCT) with azathioprine, methotrexate, or other immunosuppressive agents could not provide evidence for their use as steroid or IVIg-sparing treatments, but none of these trials was large enough to rule out a small or moderate benefit.

**3.1. Scores for Clinical Evaluation.** CIDP diagnosis should be as accurate as possible in agreement with EFNS 2010 guidelines [1]. Such criteria are quite accurate and provide a better sensitivity compared to restrictive AAN criteria [68].

Patients with very mild symptoms, not or only slightly interfering with activities of daily living, may be monitored on a yearly basis by clinical examination, nerve conduction studies, and electromyography (EMG); in selected cases, when the diagnosis is not ascertained, sural nerve biopsy might be performed.

To evaluate disability progression, several scales have been proposed. In our opinion the Rankin Score, originally proposed for stroke patients and modified in 1988, lacks sensitivity to detect mild improvement occurring in the treatment of immune-mediated polyneuropathies [69]. Therefore, to monitor disability in the follow-up setting, the INCAT Overall Disability Sum Score (ODSS) should be preferred [70]. This score covers not only mobility disturbances but also upper limb dysfunction. Moreover, it has good clinimetric properties; it captures a high proportion of variance of disability and shows a good correlation with patients' perceptions [71, 72]. A recent report suggested that the Rasch-built Overall Disability Scale, a scale that specifically captures activity and social participation limitations in patients with autoimmune demyelinating polyneuropathies, might detect ability levels better than INCAT score [73]. Other authors suggest separating the screening for motor and sensory deficits when evaluating CIDP patients, as only the motor scores correlate with CIDP disease activity status (CDAS) [74]. The CDAS is a classification focused on the long-term evolution of CIDP [75].

For muscular strength evaluation, the Medical Research Council (MRC) sum score is historically used [76], even though a recent and large study conducted on patients with neuromuscular diseases underlined possible limitations of this score and proposed a simplified and probably more reliable version, referring to only four response categories [77].

On the other side, as a pure sensory score, the INCAT Sensory Sum Score (ISS) has been proposed more than ten years ago [78]. In our experience, distal sensory deficits, with or without neuropathic pain, often persist even in aggressively treated CIDP patients. This is probably due to irreversible axonal loss, but it rarely contributes to functional disability. The strong correlation between motor scores and the disability scales could be explained by the fact that disability is mainly due to the motor impairment: patients could refer to increasing tingling and numbness without a change in the INCAT score.

Finally, the small fibres damage cannot be measured with standard EMG techniques, but it could be quantified in clinical trials or research setting with quantitative sensory testing (QST) and laser evoked potentials.

**3.2. Newly Diagnosed Patients: First-Line Treatments.** Treatment with corticosteroids or IVIg should be offered to patients with moderate or severe disability [1].

The efficacy of steroids in CIDP in the short term has been repeatedly proved, first compared to placebo [79, 80] and then to IVIg: in 2001 a controlled study has shown that a 6-week course of 60 mg daily oral prednisolone with rapid tapering is as effective as one course of IVIg at 2 g/kg [81].

If there is no major contraindication (such as diabetes or prediabetic stages as impaired fasting glucose or impaired glucose tolerance) and since it is not possible to predict if the patient will be steroid responder or not, we prefer to try steroids first because of the need of a spending review [82] and because long-term remission can be achieved in about

one-quarter of patients with CIDP after 1 or 2 courses of pulsed dexamethasone or 8-month daily prednisolone [83]. Intravenous or oral methylprednisolone [84], oral prednisolone [81], and intravenous dexamethasone [85] are all validated treatments.

However, as in other autoimmune disorders, long-term steroids as monotherapy are usually not recommended because of side effects (Cushing's syndrome, cataracts, glaucoma, diabetes, hypertension, weight gain, osteonecrosis, gastrointestinal ulcer, psychiatric disturbances, peripheral edema, hypokalemia, myopathy, and increased risk of infections).

According to literature, there is no consensus about whether to use daily or alternate-day prednisolone or prednisone or intermittent high-dose monthly intravenous or oral regimens. The generally accepted dosage for prednisolone is 60 mg/day (1-1.5 mg/kg) as induction therapy up to 12 weeks; if there is a response, the dose should be tapered to a low maintenance level over 1 or 2 years and eventually corticosteroids can be withdrawn [1]. Both daily dosing and alternate-day dosing for the oral treatment have been employed [86]. However, to our knowledge, if corticosteroids are chosen as first-line treatment intravenous pulsed therapy seems to be a more appropriate choice: the PREDICT study [85] could not show a significant difference in terms of duration of remission between pulsed high-dose dexamethasone and oral prednisolone for 6 months, but the intravenous treatment led to a faster improvement, relatively fewer relapses, and less adverse events.

Our favourite first-line therapy is methylprednisolone 500 mg IV for 4 consecutive days in the morning, every month for 6 months, but the efficacy in the short term should be equivalent to dexamethasone and prednisolone. Once IV pulsed steroid treatment shows clear cut improvement (clinical and on nerve conduction studies), an immunosuppressive agent, such as azathioprine, can be introduced in addition to oral maintenance therapy with prednisolone or prednisone at a dose of 60–80 mg/day until major improvement is seen.

Subsequently, oral steroids can be tapered, but if the patient in remission experiences a relapse, one may consider repeating the course of corticosteroids, especially if the first course led to a long-term sustained remission.

As second-line treatments two opposite approaches could be used and have equivalent effects: PE and IVIg [60].

If a patient does not respond to one of these first-line therapies, it is advisable to switch to the other one [87], but it is never clinically advisable to perform PE few days after an IVIg course.

PE leads to rapid improvement in disability, clinical impairment, and motor nerve conduction velocity in CIDP [88]; however, the main limitations of PE are the short-term benefit (usually 2 weeks) and the rate of side effects related to difficulty with venous access, use of citrate, and haemodynamic changes [89].

Repeated treatments are usually required. The optimal number of plasma exchange treatments has been reported for the acute form GBS: in one large multicenter study [90] it was shown that 2 PE are optimal for mild GBS, whereas 4 PE should be reserved for patients with moderate/severe forms.

Polyclonal human immune globulin infusion is a highly effective treatment based on multiple and still unknown mechanisms of action [43], but its cost is comparable or lower than that of PE [91] and it has fewer side effects compared to PE. The maximal clinical response to IVIg should be evident after 2 weeks from the infusion [92]. The therapeutic benefit of IVIg in CIDP in the short term was evaluated by few RCT in the 90s [93–95]. The benefit was greater for acutely relapsing patients and was reproducible after subsequent infusions [96].

In 2001 another RCT focused on 30 naïve patients and showed that IVIg is also effective as initial treatment [97].

Plasma exchange and IVIg are equally efficacious in the short term in CIDP patients [98], but PE is much less practical for maintenance treatments.

The short term efficacy of IVIg compared to placebo is supported by a large clinical trial on 117 CIDP patients called the ICE study. More than 50% of patients treated with IVIg had an improvement in the INCAT score, compared to only 20% of placebo-treated patients [55]. After this trial the use of IVIg spread over and neurologists had to consider the option of IVIg as a starting treatment [99].

The efficacy of IVIg in CIDP has been confirmed by a Cochrane review: IVIg improves disability for at least two to six weeks compared with placebo, with a number needed to treat of 3 and efficacy similar to PE or oral prednisolone [100].

In our opinion IVIg is a first-line treatment in CIDP patients with a proven contraindication to steroids and a second-line treatment or add-on treatment in patients who do not reach the expected improvement with steroids. We also prefer IVIg treatment from the beginning in patients with pure motor CIDP for whom a possible clinical worsening under steroids has been reported in a small patients series [67].

The switch from steroids to IVIg has to be considered if the patient is developing severe side effects (diabetes, osteopenia or osteonecrosis, Cushing, or cataract), if the patient is pregnant or wants to become pregnant, or if the patient is worsening more quickly than expected.

The standard dose for starting IVIg is 0.4 g/kg for 5 days (totally 2 g/kg) after checking IgA serum concentrations. The first dose is fractionated to reduce the risk of possible side effects or intolerance. Some side effects are correlated with the speed of administration; the patient should be monitored for headache, sweating, thoracic discomfort, and the dose administered over a longer time of infusion (4-5 hours).

The maximal clinical response to IVIg should be evident after 3 weeks from infusion [92]. It is advisable to schedule a follow-up visit in an outpatient setting after 1 month and after 2 months from the beginning of treatment. The second infusion of IVIg could be administered after an interval of 6 weeks from the previous infusion and during the second consult the neurologist should establish a schedule for maintenance infusions. Our standard maintenance dose is 0.5 g/day for 2 consecutive days (1 g/kg/month); a different dose should be carefully evaluated by the neurologist, according to the principle of the "lowest effective maintenance dose" [101]. Follow-up visits on a regular basis (every 4–6 months) may help to decide if the IVIg dose should be modified.

Combined treatment of steroids and IVIg from the beginning is used in other severe autoimmune diseases (e.g., myasthenia gravis), but this has to be considered an *off-label* strategy.

For patients with acute onset CIDP or with a severe form of CIDP from the beginning (INCAT score of 3 or more) we prefer treatment with IVIg or plasma exchange (with 5-6 exchanges of 1-1.5 plasma volumes over 10 days) plus steroids and an immunosuppressive agent until major improvement is noted.

**3.3. Patients Already in Follow-Up.** We always reconsider the diagnosis of CIDP if the patient does not respond to first-line treatment and in any case before starting an immunosuppressive drug, especially when there could be an underlying paraproteinemic polyneuropathy. In case of disease progression despite treatment, sural biopsy [17, 102] and repeated EMG studies could help to differentiate CIDP from other polyneuropathies from other forms.

The combination of the INCAT score plus neurophysiological follow-up could help to objectify the clinical improvement. A definition of “responder” based on disability has been suggested by Cocito and coauthors in a retrospective observational analysis [25]: responders were those patients who had an improvement of at least one point in the Rankin Scale after therapy. About 70% of patients responded to first-line immunological therapies: 61% to steroids, 73% to IVIg. Lack of response to one treatment did not preclude a response to another treatment: about 50% of the nonresponders to first-line therapy became responders when switched to an alternative drug, so that after a single switch the percentage of responders reached 80% and this proportion could be higher if treatments are combined.

Long-term treatment with corticosteroids has proved to be effective [103] but is hampered by the development of side effects that often cannot be adequately captured in short-term trials, even those with one-year follow-up. To reduce side effects, regimens alternatives to the standard 12 weeks oral prednisolone followed by 1-2 years with slow tapering have been suggested in the PREDICT study [85].

Recently, the long-term follow-up of the PREDICT study [83] provided evidence that 1-2 courses of pulsed IV dexamethasone or 8-month daily oral prednisolone allowed cure or remission in 25% of 39 CIDP patients followed up for more than 4 years.

During the monitoring period, the clinician should prevent steroid-related side effects: every patient should be provided with calcium, vitamin D, and proton-pump inhibitors. A careful monitoring of blood pressure, weight, blood sugar, and osteopenia is mandatory in all patients treated with steroids for more than 3 months.

The efficacy of long-term treatment with IVIg has been investigated in retrospective studies in comparison to PE [104] or to other treatment options in smaller [105] or larger series [25].

11 CIDP patients treated for one year with IVIg were evaluated in a neurophysiological study that showed a decrease in the rate of conduction blocks and axonal loss [106]. The most

reliable and consistent data about long-term efficacy and safety of IVIg treatment come from the extension phase of the ICE study [55]: relapse rate was significantly lower in IVIg-treated patients compared to patients who received placebo, with a side effects rate comparable between the two arms. Periodic IVIg administration significantly sustained the initial improvement seen in CIDP patients and this effect could last months without reinfusions in a significant proportion of patients. In fact, 55% of patients rerandomized to placebo did not relapse after 24 more weeks.

A recent retrospective study [107] focused on the long-term effect of IVIg in 87 Spanish patients evaluated after more than 48 weeks, with or without concomitant immunosuppressive medication. The dose of IVIg was individualized for each patient, whereas doses and frequencies were fixed in the ICE trial. The main finding of this study is that about one-quarter of patients were stable at least 6 months after the last IVIg infusion, suggesting that in the long term a careful reevaluation of the patient conditions is mandatory to avoid overtreatment and reduce costs for the healthcare system: the optimal frequency and dose of IVIg infusion should be individualized according to the patient’s need and disease course, as also stated in the EFNS guidelines [1].

Since the costs of this therapy are very high, the neurologist should find the lowest effective maintenance dose. In patients treated for years a temporary withdrawal could also be attempted: this observation time could help to decide if the patient has still, after years, a real benefit from the treatment, because patients may need less IVIg than they receive or in fact none at all. In an international study the IVIg dose could be reduced by over 20% without deterioration in almost half of the patients [108].

Some preliminary reports suggest that subcutaneous immunoglobulin may be as effective as IVIg in the maintenance therapy of CIDP [109, 110].

In 2012 IVIg and IV methylprednisolone (MP) treatments were directly compared in a multicenter, randomized, double-blind, placebo-controlled, parallel-group study [111]. This study provided evidence that the efficacy of MP is comparable to IVIg, but there are some differences concerning tolerability and effect duration. A chronic treatment with steroids is associated with a higher rate of side effects but also with longer neurological stability. Overall, almost 50% of patient from both groups did not require any further infusion after one year since they showed either improvement or symptoms stability.

Nonetheless, it should be noted that those results cannot be translated to drug naïve patients, since this study included previously treated patients.

To sustain long-term remissions there is a need for “IVIg-sparing” agents [112], as IVIg infusions are required every 3–6 weeks. In CIDP, immunosuppressive drugs such as azathioprine, cyclosporine, methotrexate, mycophenolate, and cyclophosphamide are generally used [113], but a Cochrane meta-analysis concluded that there is no evidence that they are effective [114].

Many years ago an open-label, randomized, controlled trial [115] of 27 patients, comparing azathioprine in combination with prednisone to prednisone, alone showed no

significant difference between treatments, although it should be noted that the sample size was small, the patient sample was extremely heterogeneous, and the treatment period of 9 months was too short to draw conclusions about efficacy, since several months have to pass before azathioprine reaches maximal effect.

Despite this RCT, azathioprine has been widely used in open label after initial PE or IVIg and corticosteroid treatment to maintain remission.

In patients with a long history of disease or in patients refractory to other treatments we start azathioprine at the standard dose of 2-3 mg/kg/day. This option seems to be desirable also for patients preferring a home therapy instead of a periodic access to the Day Hospital.

Data from a retrospective Italian study [116] suggest that about 25% of patients refractory to first-line treatment do respond to immunosuppressive agents (usually azathioprine or methotrexate for mild forms, cyclophosphamide for severe forms).

Oral methotrexate as a monotherapy in patients with CIDP has been compared to placebo in an RCT [108] in 60 CIDP patients who had previously responded to and were still receiving corticosteroids or IVIg. With a dose of 15 mg per week authors could not detect significant benefits, but limitations in the trial design and the high rate of responses in the placebo group meant that a treatment effect could not be excluded.

The larger study regarding cyclosporin A efficacy is based on a retrospective analysis of 19 Australian patients resistant to other therapies [117]; the efficacy of this drug is counterbalanced by kidney failure, an important side-effect.

There are few reports on the use of mycophenolate mofetil in CIDP in small series of patients with conflicting results [118–120].

Two small series of 15 [121] and 5 patients [122] treated with IV pulsed cyclophosphamide gave beneficial results, but the toxicity of this drug limits its use to refractory cases.

According to uncontrolled studies [123–125] Rituximab might be helpful in CIDP associated with hematological disorders such as monoclonal gammopathy of undetermined significance and multiple myeloma, but a very recent RCT [126] on 54 patients with anti-MAG followed up for one year has shown no significant benefit from Rituximab compared to placebo in terms of changes in the ISS score [78]. Rituximab also failed as an IVIg-sparing agent in patients dependent on IVIg [127].

Retrospective analysis based on large population studies shows that a certain proportion of CIDP patients remain free of disease in the long term, regardless of their treatment regimens; whether disease activity cessation was due to treatment effect or to spontaneous remission of the disease remains unknown [75, 107].

#### 4. Conclusions

CIDP is a rare but treatable disease. Clinical experience indicates that about 70% of patients will respond to immunomodulation; there are patients responding to steroids, whereas

others, especially with pure motor CIDP, will benefit more from IVIg or PE. It is becoming evident that CIDP is not a uniform disease but includes several variants which might have different response profiles.

First-line treatment choice depends on several factors, such as disease severity, the presence of a pure motor form of CIDP, contraindications and side effects of long-term therapy, costs, and local availability of PE or IVIg.

We would encourage international guidelines specifically devoted to define an algorithm for first-line therapy and for standardized follow-up.

There is a need to improve the identification of CIDP sub-forms not only in terms of clinical presentation (typical versus atypical) but also therapeutic response [75], especially for the IVIg treatment. It is currently under debate whether the profile of IVIg is favourable enough to outweigh the higher costs associated with its long-term use.

In conclusion, as it is mandatory to avoid overtreatment in benign forms, it is crucial to achieve long-term remission in severe forms. A short-term intensive treatment may help prevent prolonged use of corticosteroids or IVIg. In our experience, immunosuppressive agents are helpful in this long-term strategy even if, until further controlled clinical trials are available, they will remain off-label strategies.

#### Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

#### References

- [1] P. Y. K. Van Den Bergh, R. D. M. Hadden, P. Bouche et al., "European federation of neurological societies/peripheral nerve society guideline on management of chronic inflammatory demyelinating polyradiculoneuropathy: report of a joint task force of the European Federation of Neurological Societies and the Peripheral Nerve Society—first revision," *European Journal of Neurology*, vol. 17, no. 3, pp. 356–363, 2010.
- [2] H. Köller, B. C. Kieseier, S. Jander, and H.-P. Hartung, "Medical progress: chronic inflammatory demyelinating polyneuropathy," *The New England Journal of Medicine*, vol. 352, no. 13, pp. 1343–1356, 2005.
- [3] M. Odaka, N. Yuki, and K. Hirata, "Patients with chronic inflammatory demyelinating polyneuropathy initially diagnosed as Guillain-Barré syndrome," *Journal of Neurology*, vol. 250, no. 8, pp. 913–916, 2003.
- [4] A. Chiò, D. Cocito, E. Bottacchi et al., "Idiopathic chronic inflammatory demyelinating polyneuropathy: an epidemiological study in Italy," *Journal of Neurology, Neurosurgery and Psychiatry*, vol. 78, no. 12, pp. 1349–1353, 2007.
- [5] R. A. C. Hughes, D. Allen, A. Makowska, and N. A. Gregson, "Pathogenesis of chronic inflammatory demyelinating polyradiculoneuropathy," *Journal of the Peripheral Nervous System*, vol. 11, no. 1, pp. 30–46, 2006.
- [6] C. Vital, A. Vital, A. Lagueny et al., "Chronic inflammatory demyelinating polyneuropathy: immunopathological and ultrastructural study of peripheral nerve biopsy in 42 cases," *Ultrastructural Pathology*, vol. 24, no. 6, pp. 363–369, 2000.

- [7] K. V. Toyka and R. Gold, "The pathogenesis of CIDP: rationale for treatment with immunomodulatory agents," *Neurology*, vol. 60, no. 8, pp. S2–S7, 2003.
- [8] D. Allen, K. Giannopoulos, I. Gray et al., "Antibodies to peripheral nerve myelin proteins in chronic inflammatory demyelinating polyradiculoneuropathy," *Journal of the Peripheral Nervous System*, vol. 10, no. 2, pp. 174–180, 2005.
- [9] S. Kuwabara, K. Ogawara, S. Misawa, M. Mori, and T. Hattori, "Distribution patterns of demyelination correlate with clinical profiles in chronic inflammatory demyelinating polyneuropathy," *Journal of Neurology Neurosurgery and Psychiatry*, vol. 72, no. 1, pp. 37–42, 2002.
- [10] Å. Mygland, P. Monstad, and C. Vedeler, "Onset and course of chronic inflammatory demyelinating polyneuropathy," *Muscle and Nerve*, vol. 31, no. 5, pp. 589–593, 2005.
- [11] C. Bouchard, C. Lacroix, V. Planté et al., "Clinicopathologic findings and prognosis of chronic inflammatory demyelinating polyneuropathy," *Neurology*, vol. 52, no. 3, pp. 498–503, 1999.
- [12] C. Kamm and U. K. Zettl, "Autoimmune disorders affecting both the central and peripheral nervous system," *Autoimmunity Reviews*, vol. 11, no. 3, pp. 196–202, 2012.
- [13] B. C. Kieseier, H. C. Lehmann, and G. M. Z. Hörste, "Autoimmune diseases of the peripheral nervous system," *Autoimmunity Reviews*, vol. 11, no. 3, pp. 191–195, 2012.
- [14] L. J. Chi, W. H. Xu, Z. W. Zhang, H. T. Huang, L. M. Zhang, and J. Zhou, "Distribution of Th17 cells and Th1 cells in peripheral blood and cerebrospinal fluid in chronic inflammatory demyelinating polyradiculoneuropathy," *Journal of the Peripheral Nervous System*, vol. 15, no. 4, pp. 345–356, 2010.
- [15] R. Kiefer, B. C. Kieseier, G. Stoll, and H.-P. Hartung, "The role of macrophages in immune-mediated damage to the peripheral nervous system," *Progress in Neurobiology*, vol. 64, no. 2, pp. 109–127, 2001.
- [16] B. C. Kieseier, M. C. Dalakas, and H.-P. Hartung, "Immune mechanisms in chronic inflammatory demyelinating neuropathy," *Neurology*, vol. 59, no. 12, pp. S7–S12, 2002.
- [17] C. Sommer, S. Koch, M. Lammens, A. Gabreels-Festen, G. Stoll, and K. V. Toyka, "Macrophage clustering as a diagnostic marker in sural nerve biopsies of patients with CIDP," *Neurology*, vol. 65, no. 12, pp. 1924–1929, 2005.
- [18] C. Comi, M. Osio, M. Ferretti et al., "Defective Fas-mediated T-cell apoptosis predicts acute onset CIDP," *Journal of the Peripheral Nervous System*, vol. 14, no. 2, pp. 101–106, 2009.
- [19] C. Comi, "Fas-mediated T-cell apoptosis in chronic inflammatory demyelinating polyneuropathy," *Journal of the Peripheral Nervous System*, vol. 16, supplement 1, pp. 45–47, 2011.
- [20] C. Comi, T. Fleetwood, and U. Dianzani, "The role of T cell apoptosis in nervous system autoimmunity," *Autoimmunity Reviews*, vol. 12, no. 2, pp. 150–156, 2012.
- [21] G. M. Zu Hörste, H.-P. Hartung, and B. C. Kieseier, "From bench to bedside—experimental rationale for immune-specific therapies in the inflamed peripheral nerve," *Nature Clinical Practice Neurology*, vol. 3, no. 4, pp. 198–211, 2007.
- [22] T. H. Brannagan III, "Current diagnosis of CIDP: the need for biomarkers," *Journal of the Peripheral Nervous System*, vol. 16, supplement 1, pp. 3–13, 2011.
- [23] N. Latov, M. Dalakas, J.-M. Vallat, P. Donofrio, H.-P. Hartung, and K. V. Toyka, "Biomarkers in the diagnosis and therapy of CIDP: proceedings of the biomarkers faculty meeting; June 89, 2010, Athens, Greece," *Journal of the Peripheral Nervous System*, vol. 16, supplement 1, pp. 1–2, 2011.
- [24] F. T. Rotta, A. T. Sussman, W. G. Bradley, D. Ram Ayyar, K. R. Sharma, and R. T. Shebert, "The spectrum of chronic inflammatory demyelinating polyneuropathy," *Journal of the Neurological Sciences*, vol. 173, no. 2, pp. 129–139, 2000.
- [25] D. Cocito, I. Paolasso, G. Antonini et al., "A nationwide retrospective analysis on the effect of immune therapies in patients with chronic inflammatory demyelinating polyradiculoneuropathy," *European Journal of Neurology*, vol. 17, no. 2, pp. 289–294, 2010.
- [26] J. H. Austin, "Recurrent polyneuropathies and their corticosteroid treatment: with five-year observations of a placebo-controlled case treated with corticotrophin, cortisone, and prednisone," *Brain*, vol. 81, no. 2, pp. 157–192, 1958.
- [27] R. H. Oakley and J. A. Cidlowski, "Cellular processing of the glucocorticoid receptor gene and protein: new mechanisms for generating tissue-specific actions of glucocorticoids," *Journal of Biological Chemistry*, vol. 286, no. 5, pp. 3177–3184, 2011.
- [28] R. Gold, H.-P. Hartung, and H. Lassmann, "T-cell apoptosis in autoimmune diseases: termination of inflammation in the nervous system and other sites with specialized immune-defense mechanisms," *Trends in Neurosciences*, vol. 20, no. 9, pp. 399–404, 1997.
- [29] J. R. Flammer and I. Rogatsky, "Minireview: glucocorticoids in autoimmunity: unexpected targets and mechanisms," *Molecular Endocrinology*, vol. 25, no. 7, pp. 1075–1086, 2011.
- [30] U. K. Zettl, R. Gold, H.-P. Hartung, and K. V. Toyka, "Apoptotic cell death T-lymphocytes in experimental autoimmune neuritis of the Lewis rat," *Neuroscience Letters*, vol. 176, no. 1, pp. 75–79, 1994.
- [31] U. K. Zettl, R. Gold, K. V. Toyka, and H.-P. Hartung, "Intravenous glucocorticosteroid treatment augments apoptosis of inflammatory T cells in experimental autoimmune neuritis (EAN) of the Lewis rat," *Journal of Neuropathology and Experimental Neurology*, vol. 54, no. 4, pp. 540–547, 1995.
- [32] V. I. Leussink, S. Jung, U. Merschdorf, K. V. Toyka, and R. Gold, "High-dose methylprednisolone therapy in multiple sclerosis induces apoptosis in peripheral blood leukocytes," *Archives of Neurology*, vol. 58, no. 1, pp. 91–97, 2001.
- [33] E. Charmandari, G. P. Chrousos, T. Ichijo et al., "The human glucocorticoid receptor (hGR)  $\beta$  isoform suppresses the transcriptional activity of hGR $\alpha$  by interfering with formation of active coactivator complexes," *Molecular Endocrinology*, vol. 19, no. 1, pp. 52–64, 2005.
- [34] K. L. Gross, N. Z. Lu, and J. A. Cidlowski, "Molecular mechanisms regulating glucocorticoid sensitivity and resistance," *Molecular and Cellular Endocrinology*, vol. 300, no. 1–2, pp. 7–16, 2009.
- [35] J. C. Kam, S. J. Szeffler, W. Surs, E. R. Sher, and D. Y. M. Leung, "Combination IL-2 and IL-4 reduces glucocorticoid receptor-binding affinity and T cell response to glucocorticoids," *Journal of Immunology*, vol. 151, no. 7, pp. 3460–3466, 1993.
- [36] J. D. Spahn, D. Y. M. Leung, M. T. S. Chan, S. J. Szeffler, and E. W. Gelfand, "Mechanisms of glucocorticoid reduction in asthmatic subjects treated with intravenous immunoglobulin," *Journal of Allergy and Clinical Immunology*, vol. 103, no. 3, pp. 421–426, 1999.
- [37] J. F. Modiano, D. Amran, G. Lack et al., "Posttranscriptional regulation of T-cell IL-2 production by human pooled immunoglobulin," *Clinical Immunology and Immunopathology*, vol. 83, no. 1, pp. 77–85, 1997.

- [38] P. Imbach, V. d'Apuzzo, and A. Hirt, "High-dose intravenous gammaglobulin for idiopathic thrombocytopenic purpura in childhood," *The Lancet*, vol. 1, no. 8232, pp. 1228–1230, 1981.
- [39] E. W. Gelfand, "Intravenous immune globulin in autoimmune and inflammatory diseases," *The New England Journal of Medicine*, vol. 367, no. 21, pp. 2015–2025, 2012.
- [40] M. D. Kazatchkine and S. V. Kaveri, "Immunomodulation of autoimmune and inflammatory diseases with intravenous immune globulin," *The New England Journal of Medicine*, vol. 345, no. 10, pp. 747–755, 2001.
- [41] F. Nimmerjahn and J. V. Ravetch, "Anti-inflammatory actions of intravenous immunoglobulin," *Annual Review of Immunology*, vol. 26, pp. 513–533, 2008.
- [42] V.-S. Negi, S. Elluru, S. Sib  ril et al., "Intravenous immunoglobulin: an update on the clinical use and mechanisms of action," *Journal of Clinical Immunology*, vol. 27, no. 3, pp. 233–245, 2007.
- [43] M. C. Dalakas, "Mechanisms of action of IVIg and therapeutic considerations in the treatment of acute and chronic demyelinating neuropathies," *Neurology*, vol. 59, no. 12, pp. S13–S21, 2002.
- [44] M. C. Dalakas, "The use of intravenous immunoglobulin in the treatment of autoimmune neuromuscular diseases: evidence-based indications and safety profile," *Pharmacology and Therapeutics*, vol. 102, no. 3, pp. 177–193, 2004.
- [45] S. Jacob and Y. A. Rajabally, "Current proposed mechanisms of action of intravenous immunoglobulins in inflammatory neuropathies," *Current Neuropharmacology*, vol. 7, no. 4, pp. 337–342, 2009.
- [46] S. L. Katz, M. M. Frank, V. D. Miletic, and H. Jiang, "Immunoglobulin in the control of complement action," *Immunologic Research*, vol. 22, no. 2-3, pp. 137–146, 2000.
- [47] I. Schwab and F. Nimmerjahn, "Intravenous immunoglobulin therapy: how does IgG modulate the immune system?" *Nature Reviews Immunology*, vol. 13, pp. 176–189, 2013.
- [48] F. Nimmerjahn and J. V. Ravetch, "Immunology: divergent immunoglobulin G subclass activity through selective Fc receptor binding," *Science*, vol. 310, no. 5753, pp. 1510–1512, 2005.
- [49] S. Aschermann, A. Lux, A. Baerenwaldt, M. Biburger, and F. Nimmerjahn, "The other side of immunoglobulin G: suppressor of inflammation," *Clinical and Experimental Immunology*, vol. 160, no. 2, pp. 161–167, 2010.
- [50] B. Tackenberg, I. Jel  i  , A. Baerenwaldt et al., "Impaired inhibitory Fcγ receptor IIB expression on B cells in chronic inflammatory demyelinating polyneuropathy," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, no. 12, pp. 4788–4792, 2009.
- [51] Y. Kaneko, F. Nimmerjahn, and J. V. Ravetch, "Anti-inflammatory activity of immunoglobulin G resulting from Fc sialylation," *Science*, vol. 313, no. 5787, pp. 670–673, 2006.
- [52] R. Jefferis and J. Lund, "Interaction sites on human IgG-Fc for FcγR: current models," *Immunology Letters*, vol. 82, no. 1-2, pp. 57–65, 2002.
- [53] R. M. Anthony, F. Nimmerjahn, D. J. Ashline, V. N. Reinhold, J. C. Paulson, and J. V. Ravetch, "Recapitulation of IVIG anti-inflammatory activity with a recombinant IgG Fc," *Science*, vol. 320, no. 5874, pp. 373–376, 2008.
- [54] S. V. Kaveri, S. Lacroix-Desmazes, and J. Bayry, "The antiinflammatory IgG," *The New England Journal of Medicine*, vol. 359, no. 3, pp. 307–309, 2008.
- [55] R. A. Hughes, P. Donofrio, V. Bril et al., "Intravenous immune globulin (10% caprylate-chromatography purified) for the treatment of chronic inflammatory demyelinating polyradiculoneuropathy (ICE study): a randomised placebo-controlled trial," *The Lancet Neurology*, vol. 7, no. 2, pp. 136–144, 2008.
- [56] I. Gutierrez-Roelens and B. R. Lauwerys, "Genetic susceptibility to autoimmune disorders: clues from gene association and gene expression studies," *Current Molecular Medicine*, vol. 8, no. 6, pp. 551–561, 2008.
- [57] R. Gold, M. C. Dalakas, and K. V. Toyka, "Immunotherapy in autoimmune neuromuscular disorders," *The Lancet Neurology*, vol. 2, no. 1, pp. 22–32, 2003.
- [58] H. C. Lehmann, H.-P. Hartung, G. R. Hetzel, O. St  ve, and B. C. Kieseier, "Plasma exchange in neuroimmunological disorders: part 2. Treatment of neuromuscular disorders," *Archives of Neurology*, vol. 63, no. 8, pp. 1066–1071, 2006.
- [59] I. Cortese and D. R. Cornblath, "Therapeutic plasma exchange in neurology: 2012," *Journal of Clinical Apheresis*, vol. 28, pp. 16–19, 2013.
- [60] H. C. Lehmann and H.-P. Hartung, "Plasma exchange and intravenous immunoglobulins: mechanism of action in immune-mediated neuropathies," *Journal of Neuroimmunology*, vol. 231, no. 1-2, pp. 61–69, 2011.
- [61] J.-M. Vallat, C. Sommer, and L. Magy, "Chronic inflammatory demyelinating polyradiculoneuropathy: diagnostic and therapeutic challenges for a treatable condition," *The Lancet Neurology*, vol. 9, no. 4, pp. 402–412, 2010.
- [62] E. Frauger, J. Grassi, V. Pradel et al., "Use of intravenous immunoglobulins in clinical practice: data from three French university hospitals," *Fundamental and Clinical Pharmacology*, vol. 25, no. 6, pp. 753–761, 2011.
- [63] P. McCrone, D. Chisholm, M. Knapp et al., "Cost-utility analysis of intravenous immunoglobulin and prednisolone for chronic inflammatory demyelinating polyradiculoneuropathy," *European Journal of Neurology*, vol. 10, no. 6, pp. 687–694, 2003.
- [64] G. Blackhouse, K. Gaebel, F. Xie et al., "Cost-utility of Intravenous Immunoglobulin (IVIg) compared with corticosteroids for the treatment of Chronic Inflammatory Demyelinating Polyneuropathy (CIDP) in Canada," *Cost Effectiveness and Resource Allocation*, vol. 8, article 14, 2010.
- [65] M. Mahdi-Rogers, P. McCrone, and R. A. Hughes, "Economic costs and quality of life in chronic inflammatory neuropathies in southeast England," *European Journal of Neurology*, vol. 21, no. 1, pp. 34–39, 2014.
- [66] D. Cocito, G. Serra, I. Paolasso, D. A. Baril  , L. Lopiano, and L. Cattal, "Economic and quality of life evaluation of different modalities of immunoglobulin therapy in chronic dysimmune neuropathies," *Journal of the Peripheral Nervous System*, vol. 17, pp. 426–428, 2012.
- [67] L. Donaghy, K. R. Mills, S. J. Boniface et al., "Pure motor demyelinating neuropathy: deterioration after steroid treatment and improvement with intravenous immunoglobulin," *Journal of Neurology Neurosurgery and Psychiatry*, vol. 57, no. 7, pp. 778–783, 1994.
- [68] Ad Hoc Subcommittee of the American Academy of Neurology AIDS Task Force. Research criteria for diagnosis of chronic inflammatory demyelinating polyneuropathy (CIDP), "Report from an Ad Hoc Subcommittee of the American Academy of Neurology AIDS Task Force," *Neurology*, vol. 41, no. 5, pp. 617–618, 1991.
- [69] J. C. Van Swieten, P. J. Koudstaal, M. C. Visser, H. J. A. Schouten, and J. Van Gijn, "Interobserver agreement for the assessment of

- handicap in stroke patients," *Stroke*, vol. 19, no. 5, pp. 604–607, 1988.
- [70] I. S. J. Merkies, P. I. M. Schmitz, F. G. A. Van der Meché, J. P. A. Samijn, P. A. Van Doorn, and Inflammatory Neuropathy Cause and Treatment (INCAT) group, "Clinimetric evaluation of a new overall disability scale in immune mediated polyneuropathies," *Journal of Neurology Neurosurgery and Psychiatry*, vol. 72, no. 5, pp. 596–601, 2002.
- [71] I. S. J. Merkies, P. I. M. Schmitz, F. G. A. Van Der Meché, and P. A. Van Doorn, "Comparison between impairment and disability scales in immune-mediated polyneuropathies," *Muscle and Nerve*, vol. 28, no. 1, pp. 93–100, 2003.
- [72] I. S. J. Merkies and P. I. M. Schmitz, "Getting closer to patients: the INCAT Overall Disability Sum Score relates better to patients' own clinical judgement in immune-mediated polyneuropathies," *Journal of Neurology, Neurosurgery and Psychiatry*, vol. 77, no. 8, pp. 970–972, 2006.
- [73] S. I. Van Nes, E. K. Vanhoutte, P. A. Van Doorn et al., "Rasch-built Overall Disability Scale (R-ODS) for immune-mediated peripheral neuropathies," *Neurology*, vol. 76, no. 4, pp. 337–345, 2011.
- [74] P. A. Panaite, S. Renaud, R. Kraftsik, A. J. Steck, and T. Kuntzer, "Impairment and disability in 20 CIDP patients according to disease activity status," *Journal of the Peripheral Nervous System*, vol. 18, no. 3, pp. 241–246, 2013.
- [75] K. C. Gorson, I. N. Van Schaik, I. S. J. Merkies et al., "Chronic inflammatory demyelinating polyneuropathy disease activity status: recommendations for clinical research standards and use in clinical practice," *Journal of the Peripheral Nervous System*, vol. 15, no. 4, pp. 326–333, 2010.
- [76] P. J. Dyck, C. J. Boes, D. Mulder et al., "History of standard scoring, notation, and summation of neuromuscular signs. A current survey and recommendation," *Journal of the Peripheral Nervous System*, vol. 10, no. 2, pp. 158–173, 2005.
- [77] E. K. Vanhoutte, C. G. Faber, S. I. Van Nes et al., "Modifying the Medical Research Council grading system through Rasch analyses," *Brain*, vol. 135, no. 5, pp. 1639–1649, 2012.
- [78] I. S. Merkies, P. I. Schmitz, F. G. van der Meché, and P. A. van Doorn, "Psychometric evaluation of a new sensory scale in immune-mediated polyneuropathies. Inflammatory Neuropathy Cause and Treatment (INCAT) Group," *Neurology*, vol. 54, no. 4, pp. 943–949, 2000.
- [79] P. J. Dyck, P. C. O'Brien, and K. F. Oviat, "Prednisone improves chronic inflammatory demyelinating polyradiculoneuropathy more than no treatment," *Annals of Neurology*, vol. 11, no. 2, pp. 136–141, 1982.
- [80] K. C. Gorson, G. Allam, and A. H. Ropper, "Chronic inflammatory demyelinating polyneuropathy: clinical features and response to treatment in 67 consecutive patients with and without a monoclonal gammopathy," *Neurology*, vol. 48, no. 2, pp. 321–328, 1997.
- [81] R. Hughes, F. MedSci, S. Bensa et al., "Randomized controlled trial of intravenous immunoglobulin versus oral prednisolone in chronic inflammatory demyelinating polyradiculoneuropathy," *Annals of Neurology*, vol. 50, no. 2, pp. 195–201, 2001.
- [82] I. N. van Schaik, "First-line treatment for CIDP: a new piece of the puzzle," *The Lancet Neurology*, vol. 11, pp. 478–479, 2012.
- [83] F. Eftimov, M. Vermeulen, P. A. van Doorn, E. Brusse, I. N. van Schaik, and PREDICT, "Long-term remission of CIDP after pulsed dexamethasone or short-term prednisolone treatment," *Neurology*, vol. 78, pp. 1079–1084, 2012.
- [84] S. A. Muley, P. Kelkar, and G. J. Parry, "Treatment of chronic inflammatory demyelinating polyneuropathy with pulsed oral steroids," *Archives of Neurology*, vol. 65, no. 11, pp. 1460–1464, 2008.
- [85] I. N. van Schaik, F. Eftimov, P. A. van Doorn et al., "Pulsed high-dose dexamethasone versus standard prednisolone treatment for chronic inflammatory demyelinating polyradiculoneuropathy (PREDICT study): a double-blind, randomised, controlled trial," *The Lancet Neurology*, vol. 9, no. 3, pp. 245–253, 2010.
- [86] M. B. Bromberg and O. Carter, "Corticosteroid use in the treatment of neuromuscular disorders: empirical and evidence-based data," *Muscle and Nerve*, vol. 30, no. 1, pp. 20–37, 2004.
- [87] R. Nemni, S. Amadio, R. Fazio, G. Galardi, S. Previtali, and G. Comi, "Intravenous immunoglobulin treatment in patients with chronic inflammatory demyelinating neuropathy not responsive to other treatments," *Journal of Neurology Neurosurgery and Psychiatry*, vol. 57, pp. 43–45, 1994.
- [88] M. M. Mehndiratta, R. A. Hughes, and P. Agarwal, "Plasma exchange for chronic inflammatory demyelinating polyradiculoneuropathy," *Cochrane Database of Systematic Reviews*, no. 3, Article ID CD003906, 2004.
- [89] A. F. Hahn, C. F. Bolton, N. Pillay et al., "Plasma-exchange therapy in chronic inflammatory demyelinating polyneuropathy. A double-blind, sham-controlled, cross-over study," *Brain*, vol. 119, no. 4, pp. 1055–1066, 1996.
- [90] The French Cooperative Group on Plasma Exchange in Guillain-Barré Syndrome, "Appropriate number of plasma exchanges in Guillain-Barré syndrome," *Annals of Neurology*, vol. 41, pp. 298–306, 1997.
- [91] C.-P. Tsai, K.-C. Wang, C.-Y. Liu, W.-Y. Sheng, and T.-C. Lee, "Pharmacoeconomics of therapy for Guillain-Barré syndrome: plasma exchange and intravenous immunoglobulin," *Journal of Clinical Neuroscience*, vol. 14, no. 7, pp. 625–629, 2007.
- [92] N. Latov, C. Deng, M. C. Dalakas et al., "Timing and course of clinical response to intravenous immunoglobulin in chronic inflammatory demyelinating polyradiculoneuropathy," *Archives of Neurology*, vol. 67, no. 7, pp. 802–807, 2010.
- [93] M. Vermeulen, P. A. Van Doorn, A. Brand, P. F. W. Strengers, F. G. I. Jennekens, and H. F. M. Busch, "Intravenous immunoglobulin treatment in patients with chronic inflammatory demyelinating polyneuropathy: a double blind, placebo controlled study," *Journal of Neurology Neurosurgery and Psychiatry*, vol. 56, no. 1, pp. 36–39, 1993.
- [94] P. A. Van Doorn, A. Brand, P. F. W. Strengers, J. Meulstee, and M. Vermeulen, "High-dose intravenous immunoglobulin treatment in chronic inflammatory demyelinating polyneuropathy: a double-blind, placebo-controlled, crossover study," *Neurology*, vol. 40, no. 2, pp. 209–212, 1990.
- [95] T. Kubori, T. Mezaki, R. Kaji et al., "The clinical usefulness of high-dose intravenous immunoglobulin therapy for chronic inflammatory demyelinating polyneuropathy and multifocal motor neuropathy," *Brain and Nerve*, vol. 51, no. 2, pp. 127–135, 1999.
- [96] A. F. Hahn, C. F. Bolton, D. Zochodne, and T. E. Feasby, "Intravenous immunoglobulin treatment in chronic inflammatory demyelinating polyneuropathy. A double-blind, placebo-controlled, cross-over study," *Brain*, vol. 119, no. 4, pp. 1067–1077, 1996.
- [97] J. R. Mendell, R. J. Barohn, M. L. Freimer et al., "Randomized controlled trial of mg in untreated chronic inflammatory demyelinating polyradiculoneuropathy," *Neurology*, vol. 56, no. 4, pp. 445–449, 2001.

- [98] P. J. Dyck, W. J. Litchy, K. M. Kratz et al., "A plasma exchange versus immune globulin infusion trial in chronic inflammatory demyelinating polyradiculoneuropathy," *Annals of Neurology*, vol. 36, no. 6, pp. 838–845, 1994.
- [99] M. Vermeulen, "Intravenous immunoglobulin: a first-line treatment in CIDP?" *The Lancet Neurology*, vol. 7, no. 2, pp. 115–116, 2008.
- [100] F. Eftimov, J. B. Winer, M. Vermeulen, R. de Haan, and I. N. van Schaik, "Intravenous immunoglobulin for chronic inflammatory demyelinating polyradiculoneuropathy," *Cochrane Database of Systematic Reviews*, no. 1, Article ID CD001797, 2009.
- [101] Y. A. Rajabally, H. Seow, and P. Wilson, "Dose of intravenous immunoglobulins in chronic inflammatory demyelinating polyneuropathy," *Journal of the Peripheral Nervous System*, vol. 11, no. 4, pp. 325–329, 2006.
- [102] C. Sommer and K. Toyka, "Nerve biopsy in chronic inflammatory neuropathies: in situ biomarkers," *Journal of the Peripheral Nervous System*, vol. 16, no. 1, pp. 24–29, 2011.
- [103] M. M. Mehndiratta and R. A. Hughes, "Corticosteroids for chronic inflammatory demyelinating polyradiculoneuropathy," *Cochrane Database of Systematic Reviews*, no. 1, Article ID CD002062, 2002.
- [104] P. P. Choudhary and R. A. C. Hughes, "Long-term treatment of chronic inflammatory demyelinating polyradiculoneuropathy with plasma exchange or intravenous immunoglobulin," *Quarterly Journal of Medicine*, vol. 88, no. 7, pp. 493–502, 1995.
- [105] S. Kuwabara, S. Misawa, M. Mori, N. Tamura, M. Kubota, and T. Hattori, "Long term prognosis of chronic inflammatory demyelinating polyneuropathy: a five year follow up of 38 cases," *Journal of Neurology, Neurosurgery and Psychiatry*, vol. 77, no. 1, pp. 66–70, 2006.
- [106] S. Vucic, K. Black, L. E. Baldassari, P. S. Tick Chong, K. T. Dawson, and D. Cros, "Long-term effects of intravenous immunoglobulin in CIDP," *Clinical Neurophysiology*, vol. 118, no. 9, pp. 1980–1984, 2007.
- [107] L. Querol, R. Rojas-Garcia, C. Casasnovas et al., "Long-term outcome in chronic inflammatory demyelinating polyneuropathy patients treated with intravenous immunoglobulin: a retrospective study," *Muscle Nerve*, vol. 48, no. 6, pp. 870–876, 2013.
- [108] RMC Trial Group, "Randomised controlled trial of methotrexate for chronic inflammatory demyelinating polyradiculoneuropathy (RMC trial): a pilot, multicentre study," *The Lancet Neurology*, vol. 8, pp. 158–164, 2009.
- [109] D.-H. Lee, R. A. Linker, W. Paulus, C. Schneider-Gold, A. Chan, and R. Gold, "Subcutaneous immunoglobulin infusion: a new therapeutic option in chronic inflammatory demyelinating polyneuropathy," *Muscle and Nerve*, vol. 37, no. 3, pp. 406–409, 2008.
- [110] D. Cocito, G. Serra, Y. Falcone, and I. Paolasso, "The efficacy of subcutaneous immunoglobulin administration in chronic inflammatory demyelinating polyneuropathy responders to intravenous immunoglobulin," *Journal of the Peripheral Nervous System*, vol. 16, no. 2, pp. 150–152, 2011.
- [111] E. Nobile-Orazio, D. Cocito, S. Jann et al., "Intravenous immunoglobulin versus intravenous methylprednisolone for chronic inflammatory demyelinating polyradiculoneuropathy: a randomised controlled trial," *The Lancet Neurology*, vol. 11, pp. 493–502, 2012.
- [112] M. C. Dalakas, "Clinical trials in CIDP and chronic autoimmune demyelinating polyneuropathies," *Journal of the Peripheral Nervous System*, vol. 17, no. 2, pp. 34–39, 2012.
- [113] K. Kuitwaard and P. A. Van Doorn, "Newer therapeutic options for chronic inflammatory demyelinating polyradiculoneuropathy," *Drugs*, vol. 69, no. 8, pp. 987–1001, 2009.
- [114] M. Mahdi-Rogers, A. V. Swan, P. A. van Doorn, and R. A. Hughes, "Immunomodulatory treatment other than corticosteroids, immunoglobulin and plasma exchange for chronic inflammatory demyelinating polyradiculoneuropathy," *Cochrane Database of Systematic Reviews*, vol. 11, Article ID CD003280, 2010.
- [115] P. J. Dyck, P. O'Brien, and C. Swanson, "Combined azathioprine and prednisone in chronic inflammatory-demyelinating polyneuropathy," *Neurology*, vol. 35, no. 8, pp. 1173–1176, 1985.
- [116] D. Cocito, S. Grimaldi, I. Paolasso et al., "Immunosuppressive treatment in refractory chronic inflammatory demyelinating polyradiculoneuropathy. A nationwide retrospective analysis," *European Journal of Neurology*, vol. 18, no. 12, pp. 1417–1421, 2011.
- [117] M. H. Barnett, J. D. Pollard, L. Davies, and J. G. McLeod, "Cyclosporin A in resistant chronic inflammatory demyelinating polyradiculoneuropathy," *Muscle & Nerve*, vol. 21, pp. 454–460, 1998.
- [118] T. Umaphathi and R. Hughes, "Mycophenolate in treatment-resistant inflammatory neuropathies," *European Journal of Neurology*, vol. 9, no. 6, pp. 683–685, 2002.
- [119] L. Benedetti, M. Grandis, L. Nobblo et al., "Mycophenolate mofetil in dysimmune neuropathies: a preliminary study," *Muscle and Nerve*, vol. 29, no. 5, pp. 748–749, 2004.
- [120] K. C. Gorson, A. A. Amato, and A. H. Ropper, "Efficacy of mycophenolate mofetil in patients with chronic immune demyelinating polyneuropathy," *Neurology*, vol. 63, no. 4, pp. 715–717, 2004.
- [121] J. L. Good, M. Chehrena, R. F. Mayer, and C. Lee Koski, "Pulse cyclophosphamide therapy in chronic inflammatory demyelinating polyneuropathy," *Neurology*, vol. 51, no. 6, pp. 1735–1738, 1998.
- [122] D. E. Gladstone, A. A. Prestrud, and T. H. Brannagan III, "High-dose cyclophosphamide results in long-term disease remission with restoration of a normal quality of life in patients with severe refractory chronic inflammatory demyelinating polyneuropathy," *Journal of the Peripheral Nervous System*, vol. 10, no. 1, pp. 11–16, 2005.
- [123] A. Pestronk, J. Florence, T. Miller, R. Choksi, M. T. Al-Lozi, and T. D. Levine, "Treatment of IgM antibody associated polyneuropathies using rituximab," *Journal of Neurology Neurosurgery and Psychiatry*, vol. 74, no. 4, pp. 485–489, 2003.
- [124] S. Renaud, P. Fuhr, M. Gregor et al., "High-dose rituximab and anti-MAG-associated polyneuropathy," *Neurology*, vol. 66, no. 5, pp. 742–744, 2006.
- [125] L. Benedetti, C. Briani, D. Franciotta et al., "Rituximab in patients with chronic inflammatory demyelinating polyradiculoneuropathy: a report of 13 cases and review of the literature," *Journal of Neurology, Neurosurgery and Psychiatry*, vol. 82, no. 3, pp. 306–308, 2011.
- [126] J. M. Léger, K. Viala, G. Nicolas et al., "Placebo-controlled trial of rituximab in IgM anti-myelin-associated glycoprotein neuropathy," *Neurology*, vol. 80, pp. 2217–2225, 2013.
- [127] K. C. Gorson, N. Natarajan, A. H. Ropper, and R. Weinstein, "Rituximab treatment in patients with IVIg-dependent immune polyneuropathy: a prospective pilot trial," *Muscle and Nerve*, vol. 35, no. 1, pp. 66–69, 2007.

## Review Article

# An Update in Guillain-Barré Syndrome

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Guillain-Barré syndrome (GBS) was first described in 1916 (Guillain G, 1916) and is approaching its 100th anniversary. Our knowledge of the syndrome has hugely expanded since that time. Once originally considered to be only demyelinating in pathology we now recognise both axonal and demyelinating subtypes. Numerous triggering or antecedent events including infections are recognised and GBS is considered an immunological response to these. GBS is now considered to be a clinical syndrome of an acute inflammatory neuropathy encompassing a number of subtypes with evidence of different immunological mechanisms. Some of these are clearly understood while others remain to be fully elucidated. Complement fixing antibodies against peripheral nerve gangliosides alone and in combination are increasingly recognised as an important mechanism of nerve damage. New antibodies against other nerve antigens such as neurofascin have been recently described. Research databases have been set up to look at factors associated with prognosis and the influence of intravenous immunoglobulin (IVIg) pharmacokinetics in therapy. Exciting new studies are in progress to examine a possible role for complement inhibition in the treatment of the syndrome.

## 1. Introduction

Our understanding of the Guillain-Barré syndrome has improved greatly over the last decade with a much clearer idea of the clinical subtypes of the syndrome and the pathogenesis of some of the rarer variants. 2016 will mark the centenary of the original description by Guillain, Barré and Strohl [1]. They described a rapidly progressive motor disorder associated with absent reflexes and a raised CSF protein in the absence of the expected cerebrospinal fluid (CSF) pleocytosis that characterised poliomyelitis. It became clear, over the ensuing years, that the syndrome varied in severity so that in its severest form it could lead to respiratory paralysis and death [2]. Acute inflammatory demyelinating polyradiculoneuropathy (AIDP) is the most frequent subtype in the Western world with a primarily demyelinating pathology and various degrees of secondary axonal damage. Acute motor axonal neuropathy (AMAN) [3] is the next most frequent and appears to be a primary axonal disorder affecting just motor nerves. Axonal variants involving both sensory and motor nerves are much rarer Acute Motor and Sensory Axonal Neuropathy (AMSAN) [3]. Miller Fisher syndrome is generally considered to be allied to GBS although it has a uniquely tight association with anti-GQ1b antibodies.

## 2. Clinical Features

GBS has an incidence of about 1/100,000 across several studies [4, 5] in a number of countries. It increases in incidence with age and there is a small predominance of males [5].

Sensory symptoms in the legs usually mark the onset of the disease followed by rapidly progressive distal weakness that soon spreads proximally. Lumbar pain is common and may represent inflammation in the nerve roots and may coincide with the breakdown in the nerve CSF barrier that allows protein to leak into the CSF. The weakness of GBS is typically “pyramidal in distribution” with ankle dorsiflexion and knee and hip flexion often severely affected and likewise the weakness in the arms is usually more severe in shoulder abduction and elbow extension. While sensory symptoms are common sensory signs are usually minor and may be limited to loss of vibration and proprioception. The significance of reduced or absent reflexes with no objective large fibre sensory loss and yet complete paralysis leads to a frequent misdiagnosis of hysteria.

Respiratory involvement may be sudden and unexpected but usually the vital capacity falls steadily and intubation and ventilation are required at level of approximately 1 litre [6]. A small number of patients develop unusual signs such

as papilloedema [7] thought to be secondary to cerebral oedema and hyponatraemia [8]. Mild autonomic disturbance is seen in three quarters of patients but a few develop severe bradyarrhythmias which are recognised as a cause of infrequent death from the syndrome. Mortality in most population studies is between 5 and 10 percent [9]. The disease is monophasic with weakness reaching its most severity in 4 weeks followed by a plateau phase and then recovery. 60% of patients are able to walk unaided by 12 [10] months and the rest are left with various degrees of residual symptoms.

Three quarters of patients give a history of a preceding illness usually respiratory or gastrointestinal which may be so mild as to be completely asymptomatic. The neuropathy typically begins 7–10 days after any triggering infection. Numerous other antecedent events are described including surgery and immunisation. Most recent epidemiological surveys show the risk of immunisation triggering GBS to be very low [11]. It is estimated that the risk of contracting GBS from current influenza vaccines is significantly lower than the risk of getting GBS from influenza itself. Serological studies have shown that *Campylobacter jejuni*, Epstein Bar virus, and Cytomegalovirus are the most frequent antecedent infections. Patients sometimes continue to secrete *C. jejuni* in their stool for up to 3 months following the onset of GBS [12]. Persistent infection with CMV or EBV is very rare. A number of reports associate GBS with mycoplasma pneumonia, influenza, and varicella [13].

### 3. Pathology

Autopsy studies in GBS are rare because few patients die. Early studies reported oedema of the peripheral nerves with sparse inflammatory infiltrate [2]. Classic studies by Asbury and colleagues emphasised the importance of perivascular lymphocytes which resembled the findings in the animal model experimental allergic neuritis [14]. They postulated an immunological basis for the demyelination involving these lymphocytes and strongly influenced thinking about the cause of GBS. Electron microscopic studies of nerve biopsy have demonstrated macrophage associate demyelination. Macrophages appeared to invade the Schwann cell basement membrane and phagocytose myelin debris [14, 15].

Pathological studies in AMAN show a relative paucity of inflammatory infiltrate with axonal destruction but this time macrophages were situated between axons and the myelin especially in the region of the node of Ranvier [16].

The pathological studies suggest that the macrophage is the instrument of nerve damage but may well be targeted to either the myelin or axon by antibodies. In AMSAN pathological changes are similar but involve both motor and ventral nerve roots [17].

### 4. Immunology

The recognition that there was an association between GBS and a variety of triggering infections strongly suggested that there must be an immunological cause for the syndrome. This was supported by the nature of the pathological changes with

macrophage targeted, demyelination in at least AIP which could be used to support an antibody mediated disorder. The efficacy of plasma exchange in shortening the time taken to recover also argued for a serum factor mediating the disease. In the 1960's Melnick [18] was one of the first to publish data suggesting complement fixing antibodies in the acute phase of GBS. These studies were difficult to replicate but sensitive C1 esterase assays supported complement consumption and a role for complement in the disorder [19]. In rabbits immunisation with galactocerebroside can produce a demyelinating neuropathy, suggesting that antibodies against myelin antigens are capable of causing neuropathy [20]. The pathology of the human disease resembled the experimental model experimental allergic neuritis produced by immunising susceptible species with peripheral nerve in adjuvant. EAN can be elicited using individual proteins from myelin such as P0 and P2 and T cell lines reacting with P2 can transfer the disease [21, 22].

This stimulated numerous studies attempting to find antibodies to P2, P0, and other protein antigens in GBS but these were largely negative [23]. Antibodies recognising lipids were identified in the 1980's and increasingly recognised in certain subgroups of GBS [24]. The identification of antibodies against one of these gangliosides, GQ1b in 95% of patients with Miller Fisher Syndrome [25, 26], supported a role for such antibodies in the pathogenesis of this syndrome thought to be very closely related to GBS. Similar antibodies were also found in GBS with ophthalmoplegia and in Bickerstaff's encephalitis [27, 28]. In vitro studies of mouse hemidiaphragm preparations showed that antiGq1b monoclonal immunostained the neuromuscular junction where they fixed complement and bound in identical ways to patient serum [29]. Antiganglioside antibodies were found to be associated with AMAN [30] and were implicated in animal models of the disease in rabbits [31]. Furthermore, patients immunised with gangliosides [32] were known to develop neuropathies in certain circumstances adding to the body of evidence supporting a pathology for GBS which involved complement fixing antibodies against human gangliosides.

Although the evidence in support of antiganglioside antibodies as a cause of MFS and AMAN was strong the most common form of GBS on Western countries (AIDP) was only rarely associated with ganglioside antibodies using conventional techniques [33]. The frequency of antiganglioside antibodies increases if antibodies against complexes of more than one ganglioside are considered although there are as yet few published studies [34, 35]. These are eagerly awaited.

Antibodies against gangliosides are usually found to be of the IgG1 or IgG3 subtype that conventionally require T cell help in their production. T cells infiltrate the pathological lesion in GBS nerve and so it seems likely that they play a part in mediating antibody production. Several studies have identified raised concentrations of activated T cells in the peripheral blood among patients with GBS [36] as well as changes in regulatory T cells [37] and raised levels of T cell derived cytokines [38]. The early studies looking at T cell reactivity against protein antigens such as the P2 Protein which were implicated in EAN proved to be negative.  $\gamma\delta$  T cells that are capable of recognising nonprotein antigens such

as gangliosides have been isolated from GBS nerve but may be isolated from patients with vasculitis [39]. It is possible that such T cells may play a role but strong evidence is lacking.  $\gamma\delta$  T cells are restricted by CD1 which is upregulated in nerve from patients with GBS [40] but no clear CD1 polymorphism is linked to GBS [41].

The clinical features of GBS are very variable and attempts have been made to correlate this with the distribution of gangliosides in different nerves [42]. There is more GQ1b in the ocular nerves which might explain the ophthalmoplegia in Miller Fisher syndrome. Similarly ventral nerve roots contain more GM1 than dorsal roots. The actual densities and accessibilities of the gangliosides in different tissues may be more important and there are studies suggesting that access to gangliosides by antibodies may differ [43].

*C. jejuni* is the best studied triggering agent for GBS and has been shown to have ganglioside like structures in the lipopolysaccharide coat of the bacterium [44–46]. Similar examples of molecular mimicry are seen with other organisms that trigger GBS such as *Haemophilus* [47] and *Cytomegalovirus* [48]. It therefore seems plausible to hypothesise that infection with one of these agents leads to antibody production which cross-reacts with gangliosides and other glycolipids leading to myelin destruction. This could occur by complement activation or by antibodies targeting macrophages via the Fc receptor and leading to both conduction failure and demyelination.

For such specific antibodies to mediate disease they would need to pass through the blood nerve barrier. Studies in EAN suggest that activated T cells may open up the barrier to allow the antineural antibodies to mediate nerve damage [49, 50]. It is of course possible that breakdown in the blood nerve barrier is a nonspecific event that allows antigen specific antibodies to penetrate and mediate disease. Matrix metalloproteinases have been implicated in mediating barrier breakdown [51]. There may be specific factors about the triggering infection that increase the likelihood of immune sensitivity to a specific agent. Certain serotypes of *C. jejuni* appear more likely to produce these autoreactive antibodies perhaps by containing more neuritogenic epitopes [52, 53]. The risk of GBS after *C. jejuni* enteritis is estimated to be about 1 in 1000. This risk must be influenced by immunological genetic factors. Studies of HLA associations with GBS are generally weak [54, 55]. Only a very small number of familial cases of GBS have been described [56, 57].

Although antiganglioside antibodies are the most commonly reported antibody in GBS there are other reports of antibodies that might be pathogenic in a small number of patients. Antibodies against a protein in the node of Ranvier “neurofascin” have received recent attention with serum of 4% of patients with AIDP being positive in one recent study [58].

## 5. Neurophysiology

Neurophysiology is extremely useful in the diagnosis and definition of the subtype of GBS. Assessment early in the course of the syndrome frequently shows small action potentials, prolonged distal motor latency, delayed F waves,

and conduction block [59]. Occasionally the first study is normal and a repeat study is required to document a peripheral nerve disorder. Axonal forms of the disease are characterised by reduced motor and/or sensory action potentials with denervation potentials once the acute stage of the disease is over. Neurophysiological studies carried out as part of the European IvIg and steroid trial found 69% of the studies to be consistent with AIDP with only 3% suggesting axonal pathology on studies carried out within 3 weeks of onset. Twenty-three percent of studies were equivocal at this early stage and may have gone on to be predominantly axonal [60].

## 6. Management

Supportive aspects of management have been the major factor in improving mortality in GBS with the advent of good ITU care and modern methods of ventilation. Infection, emboli, and autonomic instability are the major causes of death. Passive movement of limbs and active physiotherapy once the initial acute stage is over appear to be beneficial although it has never been subject to a controlled clinical trial.

Active immune modulation with IvIg [61] or plasma exchange [62] is the mainstay of treatment with IvIg being preferred in most circumstances due to ease of availability and greater safety in patients with unstable blood pressure and pulse. IvIg is usually given at a dose of 0.4 gm/kg for 5 days although the optimum dose has never been established. Recent studies suggest that metabolism of IvIg is faster in patients with a worse prognosis and there are studies in place to see whether a higher dose of IvIg would benefit some patients [63].

Patients that either fail to improve or exhibit a deterioration are often given a further course of IvIg although trials have yet to justify such an approach. The combination of IvIg with either steroids or plasma exchange seems to confer little benefit [64].

Better treatments of GBS are clearly needed to reduce the proportion of patients that are left disabled. Complement inhibitors such as eculizumab have been shown to be effective in animal models of Miller Fisher syndrome [65] and to be safe in man [66] but have yet to be the subject of a controlled trial. Since much of the damage to nerves occurs early in the course of the disease it may be more effective to look at chemicals capable of improving nerve regrowth and regeneration. Such neuroprotective drugs would clearly be of value in a number of diseases with a common end point of axonal damage.

## Conflict of Interests

The author declares that there is no conflict of interests regarding the publication of this paper.

## References

- [1] G. Guillain, J. Barré, and A. Strohl, “Sur un syndrome de radiculo-nevrite avec hyperalbuminose du liquide cephalo-rachidien sans reaction cellulaire. Remarques sur les caracteres

- clinique et graphique des reflexes tendinaux," *Bulletins et Memoires de la Societe Medicale des Hopitaux de Paris*, vol. 40, pp. 1462–1470, 1916.
- [2] W. K. J. Haymaker, "The Landry-Guillain-Barré syndrome: a clinicopathologic report of fifty fatal cases and a critique of the literature," *Medicine*, vol. 28, pp. 59–141, 1949.
  - [3] J. W. Griffin, C. Y. Li, T. W. Ho et al., "Guillain-Barré syndrome in northern China. The spectrum of neuropathological changes in clinically defined cases," *Brain*, vol. 118, no. 3, pp. 577–595, 1995.
  - [4] A. McGrogan, G. C. Madle, H. E. Seaman, and C. S. De Vries, "The epidemiology of Guillain-Barré syndrome worldwide: a systematic literature review," *Neuroepidemiology*, vol. 32, no. 2, pp. 150–163, 2009.
  - [5] J. J. Sejvar, A. L. Baughman, M. Wise, and O. W. Morgan, "Population incidence of Guillain-Barré syndrome: a systematic review and meta-analysis," *Neuroepidemiology*, vol. 36, no. 2, pp. 123–133, 2011.
  - [6] N. D. Lawn, D. D. Fletcher, R. D. Henderson, T. D. Wolter, and E. F. M. Wijdicks, "Anticipating mechanical ventilation in Guillain-Barré syndrome," *Archives of Neurology*, vol. 58, no. 6, pp. 893–898, 2001.
  - [7] A. C. Reid and I. T. Draper, "Pathogenesis of papilloedema and raised intracranial pressure in Guillain-Barré syndrome," *British Medical Journal*, vol. 281, no. 6252, pp. 1393–1394, 1980.
  - [8] B. M. Colls, "Guillain-Barré syndrome and hyponatraemia," *Internal Medicine Journal*, vol. 34, no. 4, p. 218, 2004.
  - [9] N. Souayah, A. Nasar, M. F. K. Suri, and A. I. Qureshi, "National trends in hospital outcomes among patients with Guillain-Barré syndrome requiring mechanical ventilation," *Journal of Clinical Neuromuscular Disease*, vol. 10, no. 1, pp. 24–28, 2008.
  - [10] J. B. Winer, R. A. C. Hughes, and C. Osmond, "A prospective study of acute idiopathic neuropathy. I. Clinical features and their prognostic value," *Journal of Neurology Neurosurgery & Psychiatry*, vol. 51, no. 5, pp. 605–612, 1988.
  - [11] C. Bardage, I. Persson, A. Ortqvist, U. Bergman, J. F. Ludvigsson, and F. Granath, "Neurological and autoimmune disorders after vaccination against pandemic influenza A (H1N1) with a monovalent adjuvanted vaccine: population based cohort study in Stockholm, Sweden," *BMJ*, vol. 343, p. d5956, 2011.
  - [12] E. A. Goddard, A. J. Lastovica, and A. C. Argent, "Campylobacter 0:4:1 isolation in Guillain-Barré syndrome," *Archives of Disease in Childhood*, vol. 76, no. 6, pp. 526–528, 1997.
  - [13] F. Cresswell, J. Eadie, N. Longley, and D. Macallan, "Severe Guillain-Barré syndrome following primary infection with varicella zoster virus in an adult," *International Journal of Infectious Diseases*, vol. 14, no. 2, pp. e161–e163, 2010.
  - [14] A. K. Asbury, B. G. Arnason, and R. D. Adams, "The inflammatory lesion in idiopathic polyneuritis. Its role in pathogenesis," *Medicine*, vol. 48, no. 3, pp. 173–215, 1969.
  - [15] J. W. Prineas, "Acute idiopathic polyneuritis. An electron microscope study," *Laboratory Investigation*, vol. 26, no. 2, pp. 133–147, 1972.
  - [16] J. W. Griffin, C. Y. Li, C. Macko et al., "Early nodal changes in the acute motor axonal neuropathy pattern of the Guillain-Barré syndrome," *Journal of Neurocytology*, vol. 25, no. 1, pp. 33–51, 1996.
  - [17] J. W. Griffin, C. Y. Li, T. W. Ho et al., "Pathology of the motor-sensory axonal guillain-barré syndrome," *Annals of Neurology*, vol. 39, no. 1, pp. 17–28, 1996.
  - [18] S. C. Melnick, "Thirty-eight cases of the Guillain-Barré syndrome: an immunological study," *British Medical Journal*, vol. 1, no. 5327, pp. 368–373, 1963.
  - [19] C. L. Koski, R. Humphrey, and M. L. Shin, "Anti-peripheral myelin antibody in patients with demyelinating neuropathy: quantitative and kinetic determination of serum antibody by complement component 1 fixation," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 82, no. 3, pp. 905–909, 1985.
  - [20] T. Saida, K. Saida, D. H. Silberberg, and M. J. Brown, "Experimental allergic neuritis induced by galactocerebroside," *Annals of Neurology*, vol. 9, pp. 87–101, 1981.
  - [21] H.-P. Hartung, B. Schafer, W. Fierz, K. Heininger, and K. V. Toyka, "Cyclosporin A prevents P2 T cell line-mediated experimental autoimmune neuritis (AT-EAN) in rat," *Neuroscience Letters*, vol. 83, no. 1-2, pp. 195–200, 1987.
  - [22] J. Zhu, S.-H. Pelidou, G. Deretzi et al., "P0 glycoprotein peptides 56–71 and 180–199 dose-dependently induce acute and chronic experimental autoimmune neuritis in Lewis rats associated with epitope spreading," *Journal of Neuroimmunology*, vol. 114, no. 1, pp. 90–106, 2001, [erratum appears in *Journal of Neuroimmunology*, vol. 119, no. 1, p. 150, 2001].
  - [23] R. A. C. Hughes, I. A. Gray, and N. A. Gregson, "Immune responses to myelin antigens in Guillain-Barré syndrome," *Journal of Neuroimmunology*, vol. 6, no. 5, pp. 303–312, 1984.
  - [24] R. H. Quarles, A. A. Ilyas, and H. J. Willison, "Antibodies to glycolipids in demyelinating diseases of the human peripheral nervous system," *Chemistry and Physics of Lipids*, vol. 42, no. 1–3, pp. 235–248, 1986.
  - [25] A. Chiba, S. Kusunoki, T. Shimizu, and I. Kanazawa, "Serum IgG antibody to ganglioside GQ1b is a possible marker of Miller Fisher syndrome," *Annals of Neurology*, vol. 31, no. 6, pp. 677–679, 1992.
  - [26] H. J. Willison, J. Veitch, G. Paterson, and P. G. E. Kennedy, "Miller Fisher syndrome is associated with serum antibodies to GQ1b ganglioside," *Journal of Neurology Neurosurgery & Psychiatry*, vol. 56, no. 2, pp. 204–206, 1993.
  - [27] M. Odaka, N. Yuki, and K. Hirata, "Anti-GQ1b IgG antibody syndrome: clinical and immunological range," *Journal of Neurology Neurosurgery & Psychiatry*, vol. 70, no. 1, pp. 50–55, 2001.
  - [28] M. Odaka, N. Yuki, M. Yamada et al., "Bickerstaff's brainstem encephalitis: clinical features of 62 cases and a subgroup associated with Guillain-Barré syndrome," *Brain*, vol. 126, no. 10, pp. 2279–2290, 2003.
  - [29] G. M. O'Hanlon, J. J. Plomp, M. Chakrabarti et al., "Anti-GQ1b ganglioside antibodies mediate complement-dependent destruction of the motor nerve terminal," *Brain*, vol. 124, no. 5, pp. 893–906, 2001.
  - [30] T. W. Ho, H. J. Willison, I. Nachamkin et al., "Anti-GD1a antibody is associated with axonal but not demyelinating forms of Guillain-Barré syndrome," *Annals of Neurology*, vol. 45, pp. 168–173, 1999.
  - [31] N. Yuki, M. Yamada, M. Koga et al., "Animal model of axonal Guillain-Barré syndrome induced by sensitization with GM1 ganglioside," *Annals of Neurology*, vol. 49, no. 6, pp. 712–720, 2001.
  - [32] V. Govoni, E. Granieri, M. R. Tola et al., "Exogenous gangliosides and Guillain-Barré syndrome. An observational study in the Local Health District of Ferrara, Italy," *Brain*, vol. 120, no. 7, pp. 1123–1130, 1997.

- [33] H. J. Willison and C. S. Goodyear, "Glycolipid antigens and autoantibodies in autoimmune neuropathies," *Trends in Immunology*, vol. 34, no. 9, pp. 453–459, 2013.
- [34] K. Kaida and S. Kusunoki, "Antibodies to gangliosides and ganglioside complexes in Guillain-Barré syndrome and Fisher syndrome: mini-review," *Journal of Neuroimmunology*, vol. 223, no. 1-2, pp. 5–12, 2010.
- [35] S. Kusunoki, K.-I. Kaida, and M. Ueda, "Antibodies against gangliosides and ganglioside complexes in Guillain-Barré syndrome: new aspects of research," *Biochimica et Biophysica Acta*, vol. 1780, no. 3, pp. 441–444, 2008.
- [36] W. Hu, A. Janke, S. Ortler et al., "Expression of CD28-related costimulatory molecule and its ligand in inflammatory neuropathies," *Neurology*, vol. 68, no. 4, pp. 277–282, 2007.
- [37] L.-J. Chi, H.-B. Wang, Y. Zhang, and W.-Z. Wang, "Abnormality of circulating CD4<sup>+</sup>CD25<sup>+</sup> regulatory T cell in patients with Guillain-Barré syndrome," *Journal of Neuroimmunology*, vol. 192, no. 1-2, pp. 206–214, 2007.
- [38] K. K. Nyati, K. N. Prasad, A. Verma, and V. K. Paliwal, "Correlation of matrix metalloproteinases-2 and -9 with proinflammatory cytokines in Guillain-Barré syndrome," *Journal of Neuroscience Research*, vol. 88, no. 16, pp. 3540–3546, 2010.
- [39] A. Ben-Smith, J. S. H. Gaston, P. C. Barber, and J. B. Winer, "Isolation and characterisation of T lymphocytes from sural nerve biopsies in patients with Guillain-Barré syndrome and chronic inflammatory demyelinating polyneuropathy," *Journal of Neurology Neurosurgery & Psychiatry*, vol. 61, no. 4, pp. 362–368, 1996.
- [40] A. Khalili-Shiraz, N. Gregson, and R. Hughes, "CD 1 expression in human peripheral nerve of GBS patients," *Biochemical Society Transactions*, vol. 25, no. 2, p. 172, 1997.
- [41] M. L. Kuijff, K. Geleijns, N. Ennaji, W. van Rijs, P. A. van Doorn, and B. C. Jacobs, "Susceptibility to Guillain-Barré syndrome is not associated with CD1A and CD1E gene polymorphisms," *Journal of Neuroimmunology*, vol. 205, no. 1-2, pp. 110–112, 2008.
- [42] A. Chiba, S. Kusunoki, H. Obata, R. Machinami, and I. Kanazawa, "Ganglioside composition of the human cranial nerves, with special reference to pathophysiology of Miller Fisher syndrome," *Brain Research*, vol. 745, no. 1-2, pp. 32–36, 1997.
- [43] Y. Gong, Y. Tagawa, M. P. T. Lunn et al., "Localization of major gangliosides in the PNS: implications for immune neuropathies," *Brain*, vol. 125, no. 11, pp. 2491–2506, 2002.
- [44] C. W. Ang, H. P. Endtz, B. C. Jacobs et al., "Campylobacter jejuni lipopolysaccharides from Guillain-Barré syndrome patients induce IgG anti-GM1 antibodies in rabbits," *Journal of Neuroimmunology*, vol. 104, no. 2, pp. 133–138, 2000.
- [45] K. A. Sheikh, I. Nachamkin, T. W. Ho et al., "Campylobacter jejuni lipopolysaccharides in Guillain-Barré syndrome: molecular mimicry and host susceptibility," *Neurology*, vol. 51, no. 2, pp. 371–378, 1998.
- [46] N. Yuki, "Molecular mimicry between gangliosides and lipopolysaccharides of *Campylobacter jejuni* isolated from patients with Guillain-Barré syndrome and Miller Fisher syndrome," *Journal of Infectious Diseases*, vol. 176, no. 6, pp. S150–S153, 1997.
- [47] M. Mori, S. Kuwabara, M. Miyake et al., "Haemophilus influenzae has a GM1 ganglioside-like structure and elicits Guillain-Barré syndrome," *Neurology*, vol. 52, no. 6, pp. 1282–1284, 1999.
- [48] A. Khalili-Shirazi, N. Gregson, I. Gray, J. Rees, J. Winer, and R. Hughes, "Antiganglioside antibodies in Guillain-Barré syndrome after a recent cytomegalovirus infection," *Journal of Neurology Neurosurgery & Psychiatry*, vol. 66, no. 3, pp. 376–379, 1999.
- [49] J. M. Spies, J. D. Pollard, J. G. Bonner, K. W. Westland, and J. G. McLeod, "Synergy between antibody and P2-reactive T cells in experimental allergic neuritis," *Journal of Neuroimmunology*, vol. 57, no. 1-2, pp. 77–84, 1995.
- [50] K. W. Westland, J. D. Pollard, S. Sander, J. G. Bonner, C. Linington, and J. G. McLeod, "Activated non-neural specific T cells open the blood-brain barrier to circulating antibodies," *Brain*, vol. 122, no. 7, pp. 1283–1291, 1999.
- [51] A. Créange, T. Sharshar, T. Planchenault et al., "Matrix metalloproteinase-9 is increased and correlates with severity in Guillain-Barré syndrome," *Neurology*, vol. 53, no. 8, pp. 1683–1691, 1999.
- [52] M. Koga, M. Gilbert, J. Li et al., "Antecedent infections in Fisher syndrome: a common pathogenesis of molecular mimicry," *Neurology*, vol. 64, no. 9, pp. 1605–1611, 2005.
- [53] N. Yuki, T. Taki, M. Takahashi et al., "Penner's serotype 4 of *Campylobacter jejuni* has a lipopolysaccharide that bears a GM1 ganglioside epitope as well as one that bears a GD1a epitope," *Infection and Immunity*, vol. 62, no. 5, pp. 2101–2103, 1994.
- [54] J. B. Winer, D. Briggs, K. Welsh, and R. A. C. Hughes, "HLA antigens in the Guillain-Barré syndrome," *Journal of Neuroimmunology*, vol. 18, no. 1, pp. 13–16, 1988.
- [55] J. H. Rees, R. W. Vaughan, E. Kondeatis, and R. A. C. Hughes, "HLA-class II alleles in Guillain-Barré syndrome and miller fisher syndrome and their association with preceding *Campylobacter jejuni* infection," *Journal of Neuroimmunology*, vol. 62, no. 1, pp. 53–57, 1995.
- [56] G. A. MacGregor, "Familial Guillain-Barré syndrome," *The Lancet*, vol. 2, no. 7425, p. 1296, 1965.
- [57] M. Saunders and M. Rake, "Familial Guillain-Barré syndrome," *The Lancet*, vol. 286, no. 7422, pp. 1106–1107, 1965.
- [58] J. K. Ng, J. Malotka, N. Kawakami et al., "Neurofascin as a target for autoantibodies in peripheral neuropathies," *Neurology*, vol. 79, pp. 2241–2248, 2012.
- [59] D. R. Cornblath, "Electrophysiology in Guillain-Barré syndrome," *Annals of Neurology*, vol. 27, pp. S17–S20, 1990.
- [60] R. D. M. Hadden, D. R. Cornblath, R. A. C. Hughes et al., "Electrophysiological classification of Guillain-Barré syndrome: clinical associations and outcome," *Annals of Neurology*, vol. 44, no. 5, pp. 780–788, 1998.
- [61] R. A. Hughes, J. C. Raphaël, A. V. Swan, and P. A. van Doorn, "Intravenous immunoglobulin for Guillain-Barré syndrome," *Cochrane Database of Systematic Reviews*, no. 1, Article ID CD002063, 2006.
- [62] J. C. Raphaël, S. Chevret, R. A. Hughes, and D. Annane, "Plasma exchange for Guillain-Barré syndrome," *Cochrane Database of Systematic Reviews*, no. 2, Article ID CD001798, 2001.
- [63] K. Kuitwaard, J. De Gelder, A. P. Tio-Gillen et al., "Pharmacokinetics of intravenous immunoglobulin and outcome in Guillain-Barré syndrome," *Annals of Neurology*, vol. 66, no. 5, pp. 597–603, 2009.
- [64] Anonymous, "Randomised trial of plasma exchange, intravenous immunoglobulin, and combined treatments in Guillain-Barré syndrome," *The Lancet*, vol. 349, no. 9047, pp. 225–230, 1997.
- [65] S. K. Halstead, P. D. Humphreys, F. M. P. Zitman, J. Hamer, J. J. Plomp, and H. J. Willison, "C5 inhibitor rEV576 protects against neural injury in an in vitro mouse model of Miller Fisher

syndrome," *Journal of the Peripheral Nervous System*, vol. 13, no. 3, pp. 228–235, 2008.

- [66] A. M. Fitzpatrick, C. A. Mann, S. Barry, K. Brennan, J. R. Overell, and H. J. Willison, "An open label clinical trial of complement inhibition in multifocal motor neuropathy," *Journal of the Peripheral Nervous System*, vol. 16, no. 2, pp. 84–91, 2011.

## Review Article

# Current Understanding on the Role of Standard and Immunoproteasomes in Inflammatory/Immunological Pathways of Multiple Sclerosis

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The ubiquitin-proteasome system is the major intracellular molecular machinery for protein degradation and maintenance of protein homeostasis in most human cells. As ubiquitin-proteasome system plays a critical role in the regulation of the immune system, it might also influence the development and progression of multiple sclerosis (MS). Both *ex vivo* analyses and animal models suggest that activity and composition of ubiquitin-proteasome system are altered in MS. Proteasome isoforms endowed of immunosubunits may affect the functionality of different cell types such as CD8<sup>+</sup> and CD4<sup>+</sup> T cells and B cells as well as neurons during MS development. Furthermore, the study of proteasome-related biomarkers, such as proteasome antibodies and circulating proteasomes, may represent a field of interest in MS. Proteasome inhibitors are already used as treatment for cancer and the recent development of inhibitors selective for immunoproteasome subunits may soon represent novel therapeutic approaches to the different forms of MS. In this review we describe the current knowledge on the potential role of proteasomes in MS and discuss the *pro et contra* of possible therapies for MS targeting proteasome isoforms.

## 1. Multiple Sclerosis and Proteasome Isoforms

Multiple sclerosis (MS) is a chronic disease of the central nervous system (CNS) characterized by the presence of inflammation, myelin damage, and axonal degeneration. There are two main clinical courses of multiple sclerosis: about 90% of MS patients experience the relapsing-remitting MS phase (RRMS), characterized by disability episodes followed by a complete or partial recovery. Multifocal lesions are found by magnetic resonance imaging, typically but not

exclusively, in the white matter of the optic nerve, brain stem, cerebellum, and spinal cord. Some lesions are enhanced after intravenous administration of gadolinium, indicating breakdown of the blood-brain barrier (BBB) as a result of active inflammation. The majority of RRMS patients enter into a secondary progressive phase (SPMS), characterized by a variable degree of inflammation and a continuous and progressive neurological decline in disability state (with or without superimposed relapses) [1, 2]. A minor percentage (10%) of MS patients shows a primary progressive form of MS

(PPMS), characterized by progression of neurological disability from onset. Clinically relevant factors differentiating RRMS and PPMS are age at disease onset (a decade later in PPMS) and gender (1:1.3 male/female in PPMS versus 1:2 in RRMS) [3]. Although the initial course of RRMS and PPMS is very different, both proceed at remarkably similar rates during the progressive phase. However, it is still an ongoing debate whether the RRMS and the progressive forms of MS are the same disease observed at different stages or whether they are pathogenetically different.

One of the factors characterising MS is the autoimmune response against self-antigens and the immune-mediated demyelination which contribute, at least in part, to the neurological manifestations. Based on scientific evidence, it has been proposed that a predisposing genetic background, in combination with environmental factors such as infection, diet, sun exposure, and smoking, drives the immune system to mount an immune response towards a yet unknown myelin antigen, eventually resulting in myelin disruption [4]. Indeed, genetic associations of HLA class II (HLA-DRB1\*15) and HLA class I (HLA-A\*02, -A\*03, and -B\*07) with MS, as well as the presence of autoreactive CD4<sup>+</sup> and CD8<sup>+</sup> T lymphocytes, together with other inflammatory cells and cytokines in active MS lesions, suggest an autoimmune pathogenesis [5, 6]. Several studies support the view that an immune response in MS subjects starts and is maintained in the periphery, and specifically in the lymphatic system, although the most lethal cytotoxic effect occurs in the brain with oligodendrocytes, neuron loss, and plaque formation (outside-in model) [2]. A competing view argues that the initial malfunction occurs within the CNS, similarly to other neurodegenerative diseases, by cytodeneration, possibly focused on the oligodendrocyte-myelin complex, and a release of highly antigenic constituents that secondarily promote an autoimmune and inflammatory response in predisposed individuals [2, 7].

In the last few years, additional players have emerged in the MS pathogenic cascade, including proteasome and gut microbiota (for the latter see Section 3). The proteasome is the central catalytic unit of the ubiquitin-proteasome system, which plays several crucial functions for cell metabolism (Figure 1). By eliminating obsolete, misfolded, or aberrant proteins, the ubiquitin-proteasome system accomplishes housekeeping functions and maintains cellular homeostasis and the physiological levels of intracellular proteins. It has been demonstrated that proteasome inactivation leads to cellular death by apoptosis or necrosis [8–10]. The central role of ubiquitin-proteasome system in inflammatory responses is supported by evidence of its involvement in the on/off switching of many cellular pathways through the time-specific cleavage of short-life proteins, like transcription factors or molecules regulating the cell cycle [11]. Accordingly, the proteasome is crucial in several inflammatory processes by regulating cytokine signalling, cell proliferation, and clearance of potentially deleterious products of inflammation and is involved in the major histocompatibility complex (MHC) class I-mediated antigen presentation (Figure 1) [12]. Therefore, proteasome modulation can alter at different levels

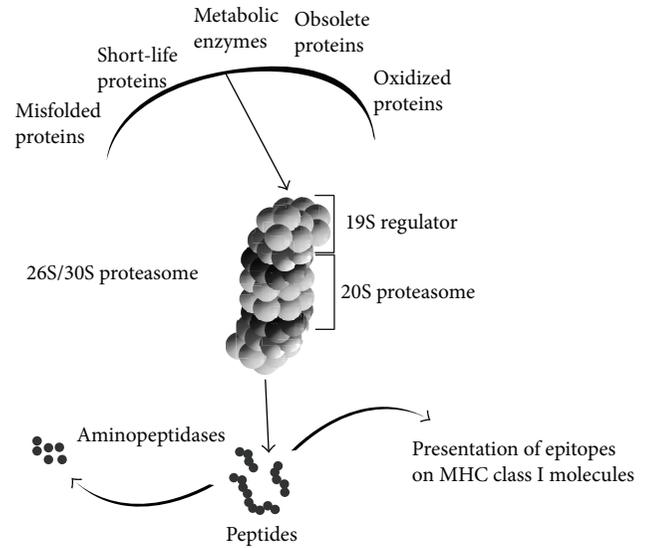


FIGURE 1: Schematic representation of the proteasome degradation pathways.

both the physiological and pathological processes of the immune system.

Different forms of proteasomes are known in eukaryotes. They vary in terms of catalytic subunits and regulatory complexes. The core 20S standard proteasome (s-proteasome) is a cylinder-shaped complex, that is, composed of four stacked rings, each consisting of seven protein subunits. Among them the  $\beta_1$ ,  $\beta_2$ , and  $\beta_5$  subunits harbour the proteolytic active sites. The result of the association of the 20S proteasome core to the PA700 regulators is the 26S/30S proteasomes, which cleave polyubiquitylated proteins in an ATP-dependent manner. 20S proteasome can also bind the PA28 regulator, which alters proteasome catalytic activities [13, 14].

The immunoproteasome (i-proteasome) is an isoform of the 20S proteasome. It carries specific catalytic subunits, that is,  $\beta_{1i}$ ,  $\beta_{2i}$ , and  $\beta_{5i}$  (also known as LMP2, MECL-1, and LMP7, resp.), which confer to the i-proteasome quantitative differences in cleavage preferences and substrate degradation rates compared to the s-proteasome. I-proteasome is generally synthesized upon interferon- $\gamma$  (IFN- $\gamma$ ) stimuli, but tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) or lipopolysaccharide has also been found to be involved in its inducible expression [15, 16]. The vast majority of endogenous peptides that are presented by the MHC class I molecules at the cell surface and recognised by CD8<sup>+</sup> T cells are generated by proteasomes. I-proteasome is generally linked to its high efficiency in the generation of the MHC class I-restricted epitopes. In support of this, i-proteasomes are predominantly expressed by professional antigen presenting cells (APCs), such as dendritic cells (DCs) and B cells, or in other cell types during inflammation, thereby indicating the i-proteasome as a major player of the MHC class I antigen presentation (Figure 1) [11, 17].

Preliminary observations on white and grey matter of MS patients suggested that the degradation rates of short

fluorogenic peptides by 20S proteasomes are decreased when compared to brain-tissue controls [18]. These results, however, cannot be interpreted as a general decrease of the proteasome-mediated proteolytic activity, as recently shown in [19–21]. Furthermore, an accumulation of i-proteasome and its regulator PA28 $\alpha\beta$  has been observed in different cell types affected by MS, such as oligodendrocytes, astrocytes, macrophages/microglia, infiltrating lymphocytes, and weakly neurons [22]. Such disease-related expression of i-proteasome is in agreement with recent observations in the experimental model of MS, that is, the experimental autoimmune encephalomyelitis (EAE). In this model, the cerebral expression of i-proteasome and PA28 $\alpha\beta$  was increased as compared with baseline levels during the acute phase of EAE. Of note, i-proteasomes were also detected in neurons, infiltrated T lymphocytes, and microglia in EAE mice [23]. However, in this study by Zheng et al., an equal expression of s- and i-proteasome subunits has been described in control mouse brain, contrasting with other studies on rodents and humans which reported a faint expression of i-proteasome in young/adult brains [21, 24, 25]. Furthermore, Zheng et al. reported no differences in the i-proteasome expression by comparing young and old control mouse brains, which is in contrast to studies on other mammals such as rats [21, 26] and humans [27], but in agreement with a study conducted on nonhuman primates [28].

The expression of i-proteasome in MS lesions or in cells involved in MS mechanisms is important because this isoform has been recently linked to different inflammatory processes. Indeed, i-proteasomes are specifically implicated in cytokine-mediated inflammation, cell growth, and differentiation in mice [11]. I-proteasome depletion alters the T cell antigen receptor (TCR) repertoire formation, the number and differentiation of CD8<sup>+</sup> T cells, and the production of proinflammatory cytokines [29]. In addition, i-proteasome depletion during IFN- $\gamma$ -mediated oxidative stress is consistent with a deficient clearance of oxidized proteins and aggregates [30, 31]. These events have been associated with worsening of EAE clinical score in  $\beta 5i^{-/-}$  mice [31], although discordant results have also been reported by others [32].

In the following sections we will discuss these and additional data suggesting an involvement of proteasomes in specific pathways underlying MS.

## 2. I-Proteasome and CD8<sup>+</sup> T Cells in MS

CD4<sup>+</sup> and CD8<sup>+</sup> T lymphocytes reactive against myelin have been found in peripheral blood, cerebrospinal fluid (CSF), and CNS plaques of MS patients, but their role in MS pathogenesis is still a matter of debate. Antimyelin CD4<sup>+</sup> T cells in MS have been widely studied because of their role in regulating cell-mediated inflammation, their ability in inducing EAE, and the identification of HLA-DRB1\*15 allele as the most significant genetic risk factor associated with MS [33]. EAE can also be triggered by the administration of CD8<sup>+</sup> T cells specific against myelin antigens in mice. In MS, CD8<sup>+</sup> T cells exceed CD4<sup>+</sup> T cells by 3–10-fold in regions of demyelination, and the degree of axonal damage

within MS lesions correlates with the number of CD8<sup>+</sup> T cells [33]. Furthermore, several studies described an increased prevalence of CD8<sup>+</sup> cytotoxic T cells reactive against specific myelin epitopes in peripheral blood of MS patients compared to healthy controls [34–36]. These observations, in addition to the genetic associations of HLA class I alleles with MS risk, suggest an involvement of CD8<sup>+</sup> T cells in MS [33].

Because i-proteasome is a major player in the processing of MHC class I-restricted epitopes in professional APCs or in inflamed conditions, it is likely that it is also involved in the presentation of myelin antigens in the MS brain. For instance, i-proteasome expression is induced in oligodendrocytes of MS patients [22]. These cells are the main producers of myelin, and hence likely to be the target of CD8<sup>+</sup> T cells in MS. Indeed, CD8<sup>+</sup> T cells were observed in close proximity to oligodendrocytes and demyelinated axons in brain tissue, towards which cytolytic granules were polarized [33]. The expression of i-proteasome in oligodendrocytes might therefore alter the presentation onto the MHC class I molecules of myelin antigens and the cytotoxic activity of specific CD8<sup>+</sup> T cells towards these cells.

Although the abovementioned scenario lacks experimental validation, there is substantial support for this theory. For instance, our group has previously observed *in vitro* that i-proteasome carrying a polymorphic variant at codon 60 (i.e., HH60) of  $\beta li$  subunit produces less amount of the myelin basic protein epitope MBP<sub>111–119</sub> [22]. This epitope is presented on the HLA-A\*02 molecule, although with moderate affinity [22] and memory CD8<sup>+</sup> cytotoxic T cells specific for this epitope are more prevalent in the blood of MS patients than controls [35–37]. We also described a lower prevalence of the  $\beta li$  HH60 variant among MS females with HLA-A\*02<sup>+</sup> genotype when compared to a matched control population. These observations led us to hypothesize that the lower risk of developing MS in HLA-A\*02<sup>+</sup> subjects carrying the  $\beta li$  HH60 variant could be—at least in part—due to a lower production of MBP<sub>111–119</sub> by oligodendrocytes or APCs in these subjects [22].

The key role of i-proteasomes in autoreactive CD8<sup>+</sup> T cell response has been recently confirmed by the observation that mice lacking i-proteasome  $\beta 5i$ - $\beta 2i$  subunits developed a multitissue autoimmune disorder mediated by CD8<sup>+</sup> T cells *via* altered MHC class I-restricted self-antigen presentation [38]. The authors of the study speculated that a relatively high percentage of MHC class I molecules present “dangerous” epitopes in presence of inflammation and in the absence of i-proteasome. These self-peptides are low-affinity binders to the MHC class I complexes (as the epitope MBP<sub>111–119</sub> [22]) and are better produced by s-proteasomes. Hence, in the absence of an appropriate i-proteasome activity these “dangerous” self-epitopes may be generated and targeted by autoreactive CD8<sup>+</sup> cytotoxic T cells, thereby triggering an autoimmune response [38]. It is attractive to hypothesize that a similar mechanism is at work in MS and would imply that i-proteasome might hamper MS development by reducing the amount of “dangerous” self-peptides presented by APCs in periphery.

Another matter of debate relies on the mechanisms causing the disruption of the immune system tolerance and

the activation of autoreactive CD4<sup>+</sup> and CD8<sup>+</sup> T lymphocytes towards CNS cells. Different studies suggest that molecular mimicry could be involved in the immune system disruption. This phenomenon describes the reaction of a single T cell clone to epitopes derived from both pathogen and human proteomes. It has been proposed that MS is triggered by a viral infection that, in the presence of (unknown) additional environmental and genetic factors, leads to an uncontrolled activation of autoreactive T cells. Such theory could explain in part the geographic distribution of the risk of developing MS [4] and is supported by several studies showing an increase of EBV-specific cellular immune responses in the blood and in the CSF of subjects with MS [5, 39–42], although the association with other viruses has also been found [4]. Conflicting results however exist about the role of molecular mimicry in driving pathological disorders associated with CD8<sup>+</sup> T cells, as a comprehensive analysis on a broad range of CD8<sup>+</sup> cytotoxic T cell clones showed a very limited number of cross-reactive T cells recognising both viral and self-epitopes [43].

The mechanisms of molecular mimicry related to CD8<sup>+</sup> T cells in autoimmune disorders could be further investigated bearing in mind another proteasome-mediated process, named proteasome-catalyzed peptide splicing (PCPS). PCPS occurs through the binding of separate peptide fragments originating from a single protein, that is, *cis*-PCPS, or from two distinct protein segments, that is, *trans*-PCPS (Figure 2) [44–46]. The role of PCPS in MS has not been investigated yet, although it might be relevant for several reasons. Firstly, PCPS is more prone to generate MHC class I-restricted potential epitopes than the simple proteasomal peptide hydrolysis because of specific biochemical features of PCPS [47]. In addition, PCPS highly increases the diversity of MHC class I-restricted epitopes from self- and viral-antigens as the number of potential peptides presented on MHC molecules is several times higher than the number of peptides encoded in the proteome [48]. Consequently, through the PCPS there could be a significant increase of MHC class I-restricted epitopes with high sequence homology to viral and human proteomes. This phenomenon implies that the activation of CD8<sup>+</sup> T cells specific for “spliced” viral epitopes with high or even complete homology with myelin antigens could represent a threat against myelin-producing cells and eventually take part in the development of MS.

### 3. Th17 Cells, Gut Microbiota, and Proteasome in MS

CD4<sup>+</sup> T cells become activated by recognising antigens presented onto the MHC class II molecules, which are only expressed on professional APCs (such as DCs, macrophages, and B cells). Upon antigen stimulus, CD4<sup>+</sup> T lymphocytes differentiate into two main subpopulations, T helper type 1 (Th1) cells and T helper type 2 (Th2) cells. Activated CD4<sup>+</sup> T cells can also differentiate into regulatory T (Treg) cells, which are characterised by the expression of the forkhead box P3 (FoxP3) transcription factor [49].

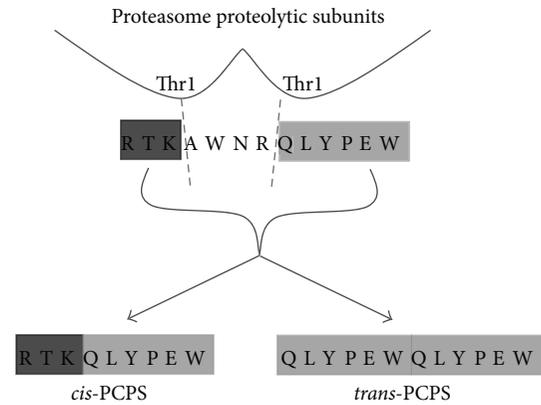


FIGURE 2: Proteasome-catalyzed peptide splicing (PCPS). PCPS can occur by ligation of two fragments of the same substrate molecule (*cis*-PCPS) or derived from two distinct protein molecules (*trans*-PCPS). Shown here are the representative cleavages (depicted by dotted lines) of the peptide gp100<sub>40–52</sub> (sequence: RTKAWN-RQLYPEW) by two distinct proteasome catalytic subunits, which generate the fragments RTK, A, WNR, and QLYPEW. According to the PCPS model [44, 47], the protein is first cleaved by the active site residue Thr1 of the proteasome proteolytic subunits, thereby producing a protein fragment. The latter peptide stays attached to the catalytic centre where, subsequently, it is ligated to a second peptide generating the proteasome-generated spliced peptide.

More recently, a new T cell subpopulation, the Th17 cells, which secretes IL-17, IL-21, and IL-22, has been described and associated with the control of extracellular pathogens [50]. Th17 cells and their cytokines are associated with several autoimmune and inflammatory diseases, such as rheumatoid arthritis, systemic lupus erythematosus, MS, psoriasis, inflammatory bowel disease, allergy, and asthma [51]. In MS patients, IL-17 expression is increased in blood mononuclear cells and in CSF as well as at the site of lesions [52]. IL-17 and IL-22 promote blood-brain barrier permeability and CNS inflammation by inducing chemokine production in endothelial cells and by downregulating tight junction proteins. IL-17 also stimulates astrocytes to produce CXC chemokines that can attract neutrophils to the BBB and activate them to release vasoactive substances [53]. It has been shown that myelin-specific Th17 cells directly interact with neuronal cells in demyelinating lesions [54]. Either deficiency or neutralization of IL-17 delay the onset and reduce the severity of EAE [55]. Furthermore, IL-23 expands Th17 cells and is critical for the induction of EAE. In contrast, a recent paper reported that overexpression of IL-2 *in vivo* reverses EAE pathology by decreasing the Th1 and Th17 infiltration. Notably, under inflammatory conditions (such as in EAE), Th17 cells display plasticity because these cells can change phenotype in inflamed tissues and secrete proinflammatory cytokines such as IFN- $\gamma$  instead of IL-17 [56].

A modifier of Th17 cell response in MS may be gut bacteria, which play an important role in shaping intestinal CD4<sup>+</sup> T cell responses [57] and in affecting brain inflammation, as suggested by evidence on gut-brain communication [58, 59].

The mammalian gastrointestinal track harbors a highly heterogeneous population of microbial organisms, which vary across geographical areas and are essential for the complete development of the immune system. The gut microbes or “microbiota” also drive a swarm of T cell responses in the gut. For instance, segmented filamentous bacteria trigger intestinal Th17 cell responses; indeed when these bacteria are used to monocolonize germ-free mice they restore Th17 cell responses in the lamina propria of the small intestine [60]. Gut bacteria are also critically involved in the differentiation of some Treg cell subsets [61] as these specific microbial organisms have developed distinct ways to promote effector T cells or Treg cell differentiation in the gut [62]. The Treg/Th17 ratio and also the Treg cell frequency have been negatively correlated with MS severity [63], thereby suggesting that the measure of their balance could be an informative biomarker for evaluating or comparing the effectiveness of MS therapies.

In the context of MS models, it has been reported that the treatment of EAE mice with probiotics reduces neuroinflammation [64] and that different gut microbiota could induce [34, 65] or tackle CNS inflammation [66]. In addition, antibiotic-mediated depletion of the gut microbiota reduces the EAE severity and the levels of proinflammatory cytokines and chemokines, whereas it increases the levels of the anti-inflammatory cytokines IL-10 and IL-13. Moreover, IL-10-producing FoxP3<sup>+</sup> Treg cells accumulate in the cervical lymph nodes of antibiotic-treated mice and protect *naïve* recipients against the transfer of EAE [65].

The tight connection between commensal gut microbiota, EAE, and Th17 lymphocytes has been recently investigated in two different models of EAE. Lee and colleagues [67] studied the induction of EAE by immunizing germ-free bacteria, specific-pathogen-free and control mice with MOG<sub>35–55</sub> peptide + *Mycobacterium tuberculosis*. They observed that germ-free mice are highly resistant to EAE development and have a lower prevalence of Th17 and Th1 cells leading to the conclusion that there is a hampering of the systemic and neuronal proinflammatory Th17 and Th1 response during EAE in absence of commensal microbiota in mice. This phenomenon seems to be reversible because intestinal colonization with segmented filamentous bacteria in germ-free mice promotes EAE development. They concluded that the microbiota dynamically and reversibly impacts the programming of pathogenic immune response during autoimmunity and that microbial colonization may provide proinflammatory signals that affect the reciprocal development of Th and Treg cells both in gut and in CNS [67].

In a second article, Berer and colleagues [68] reported that germ-free mice develop less frequently EAE, a phenomenon accompanied by a reduced number of Th17 cells in the lamina propria and reduced secretion of IL-17 and IFN- $\gamma$  by splenic T cells in response to cognate antigen stimulation. In this latter study, a spontaneous remitting-relapsing EAE mouse model has been used. These mice express, in a large proportion of their CD4<sup>+</sup> T cells, a transgenic TCR that recognizes MOG<sub>92–106</sub> peptide in the context of MHC class II molecules [68]. The fact that two independent studies described, in different models of EAE, an impairment of Th17-mediated induction of EAE in germ-free mice supports

the hypothesis that gut microbiota may influence MS *via* Th17 cell activity.

Another modifier of the Th17 cell response in MS may be the i-proteasome. Indeed, it has been shown that the *in vitro* administration of i-proteasome  $\beta$ 5i subunit inhibitor prevents the early activation of CD4<sup>+</sup> T cells, their differentiation into Th17 cells, and the secretion of TNF- $\alpha$ , IL-23, and IL-6 [69]. *In vivo*,  $\beta$ 5i inhibition or deficiency results in reduced Th1 and Th17 cell expansion and Treg cell development through STAT3/STAT1/SMAD phosphorylation [70]. The treatment with  $\beta$ 5i subunit inhibitor also attenuates the progression of the experimental arthritis in mice [69]. Because this phenomenon acts on the Th17 differentiation pathway and it is not observed by inhibiting s-proteasome activity, we may speculate that a selective block of i-proteasome  $\beta$ 5i subunit in mice might also tackle the development of EAE. Preliminary evidence in dextran sodium sulfate-induced colitis indirectly support such speculation, since this animal model mimics inflammatory bowel diseases, such as Crohn's disease and ulcerative colitis, which are characterized by a marked mucosal infiltration of T cells that secrete Th1 and Th17 cytokines and alterations of faecal and mucosal bacterial communities [71]. Interestingly, in  $\beta$ 5i subunit  $-/-$  mice and in wild type mice treated with a proteasome inhibitor, there is a reduction in the secretion of proinflammatory cytokines and chemokines, the infiltration into the colon by neutrophils, and the expansion of Th1 and Th17 cells, thereby preventing excessive tissue damage [72]. These observations are in agreement with the results of Basler et al. [73], which showed a role of  $\beta$ 5i subunit inhibition in reducing the production of proinflammatory cytokines, inflammation, tissue destruction, and consequently pathological symptoms of experimental colitis.

These data suggest that in EAE the activity of Th17 cells could be regulated by gut microbiota and i-proteasomes. Therefore, both of them may be potential targets for the treatment of MS, although there are no studies that investigated the direct interaction between gut microbiota and i-proteasome in EAE.

#### 4. Humoral Immunity, Proteasomes, and MS

The understanding of MS pathogenesis has been mostly driven by studies on T cells and their inflammatory cytokines produced in damaged tissues [74]. The interest regarding the antibody-dependent as well as antibody-independent B cell involvement has received a strong boost from the success of clinical trials targeting B cells in MS and other autoimmune diseases [75, 76].

Beyond their ability to produce antibodies, B cells function as APC, thereby contributing to T cells activation in the CNS [77]. They also influence the immune response through the production of effector cytokines, such as those involved in immune regulation (e.g., anti-inflammatory IL-10), polarization (IL-4), and cytokines involved in lymphoid tissue organization (e.g., TNF- $\alpha$  and leukotrienes) [78]. Remarkably, decreased levels of IL-10 and increased concentrations of TNF- $\alpha$  and leukotrienes have been described in

patients affected by MS [79], thus contributing to abnormal T-cell activation. This fact provides a conceivable mechanism of action to explain why B cell depletion may be relevant, both in the periphery and in the CNS, in diminishing new MS activity [79]. Indeed, Rituximab, a monoclonal antibody against CD20 molecules, exerts its therapeutic effect through a rapid and profound depletion of peripheral B cell, along with a significant reduction in the volume of T2 lesions and clinical relapse in the RRMS patients, and a reduced disease progression in PPMS [80, 81]. Additionally, in a small cohort of PPMS, it has been shown that Rituximab temporarily suppresses the activation of B cells in CSF [82]. However, the presence of regulatory B cell subsets (B regs), which could either induce or inhibit immune response, accounts for the variable effects that targeting B cells may have *in vivo* [77–80]. At present, new monoclonal antibodies (i.e., Ocrelizumab, Ofatumumab) targeting CD20 or specific surface markers of B cell subset (i.e. Atacicept) are under investigation in phase II/II trials [83, 84].

Although at present there is no data available on proteasome isoforms, B cell regulation, and MS, the recent observation of Hensley et al. [85] is relevant to connect all these three topics. Indeed, the authors reported that i-proteasome  $\beta$ 1i subunit  $-/-$  mice have a defect in B cells maturation and Ig isotope switch upon viral infection as well as in CD4<sup>+</sup> T cell survival and DC activation. They identified in the NF- $\kappa$ B activation one of the pathways affected by the presence of intermediate type proteasomes instead of the i-proteasome, which is normally present in these cells. A role of i-proteasome in modulating NF- $\kappa$ B signalling has also been observed by Maldonado and coworkers [86] in retinal pigment epithelial cells of  $\beta$ 1i subunit  $-/-$  mice. In knockout mice a higher content and a diminished activation of the NF- $\kappa$ B alternative pathway, as well as a delayed termination of the classical pathway, after *in vitro* stimulation by TNF- $\alpha$ , has been observed compared to wild type littermates [86].

Concerning the role and significance of antibodies in MS patients, the presence of CSF oligoclonal bands and increased immunoglobulin IgG synthesis is a frequent feature of MS [87] as well as other localized autoimmune diseases of the CNS [88]. These pathogenic autoantibodies (autoAbs) can induce tissue damage and thus be involved in plaque initiation and demyelination by recruiting macrophages and by complement deposition in white matter lesion of MS patients [89]. However, the antigenic targets of these antibodies and their potential use as biomarkers of MS are still a matter of debate. Indeed, autoAbs against antigens not specific for the CNS have also been associated with MS, although it is unclear if they are pathogenic effectors instead of being secondary products of the release of antigens upon CNS tissue damage. Proteasome Abs, for example, are elevated in sera of RR-, PP-, and SP-MS patients compared to other autoimmune diseases or healthy controls [90–92]. It has been shown *in vitro* that autoAbs against 20S proteasome block the proteasome activation by PA28 regulator, thereby suggesting that these autoAbs might have a regulatory function towards extracellular proteasomes such as circulating proteasomes [93]. Notably, although proteasomes are mainly studied as intracellular proteases, extracellular circulating proteasomes

are normally present in peripheral blood, and their levels are significantly increased in a variety of pathological conditions, including autoimmune diseases and tumours [94]. In particular, as biomarkers of ongoing pathological mechanisms, circulating proteasomes have demonstrated to have prognostic power as regards therapy outcome and survival in multiple myeloma patients [95]. Although cells originating extracellular proteasomes detected in peripheral blood and in the CSF have not been identified, an active release of circulating proteasomes has been recently proposed [96] as they have been copurified with exosomes [97]. In line with this hypothesis, the immunological activity rather than the cellular damage has been suggested as the causative mechanism for increased circulating proteasome levels in sepsis and severe injury [98]. Recently, a preliminary study carried out on a limited number of patients affected by RRMS has shown that circulating proteasome amount increases in MS and even further in MS patients treated with IFN- $\beta$ . The authors have also described a specific proteasome activity pattern in plasma of MS patients although they have not reported appropriate control experiments with proteasome inhibitors [99]. This preliminary observation, however, might be relevant for future studies. Indeed, a fascinating speculation is that circulating proteasomes in peripheral blood are not only simple biomarkers of inflammatory status, but also active proteases that might control cytokine levels, cell-mediated cytotoxicity, and plasma membrane permeability [94] and synergize with other component to ameliorate tissue damage [97].

## 5. Maintenance of Cellular Homeostasis during Inflammation-Mediated Oxidative Stress in MS

The pathological mechanisms of neurodegeneration, although largely unknown, are often mediated by oxidative stress and excitotoxicity (degenerative cascade), two processes that are closely interactive [100, 101]. The increased production of reactive oxygen and nitrogen species induces oxidative damage to different cellular components including lipid, DNA, and proteins [102]. Accordingly, in MS patients, oxidized DNA is present in a small number of reactive astrocytes as well as in oligodendrocyte nuclei, with evidence of apoptosis [103]. Similarly, lipid peroxidation-derived structures (malondialdehyde and oxidized phospholipid epitopes) can be detected in the cytoplasm of oligodendrocytes and some astrocytes as well as in degenerating neurons within grey matter lesions [103]. Oxidized proteins are more prevalent in cerebellar astrocytes as well as in spinal cord neurons of EAE mice [104, 105]. In such scenario, an effective removal of oxidized proteins seems to be a key element to maintain cellular homeostasis during neuroinflammation.

Studies performed on neuronal cell lines have suggested that proteasome plays a central role in mitochondria homeostasis. Proteasome inhibition decreases the activity of complexes I and II and increases the production of reactive oxygen species and the accumulation of lipofuscin,

a highly oxidized cross-linked aggregate of oxidized protein and lipid [106, 107]. In addition, proteasome is essential in maintaining cell homeostasis by degrading obsolete, damaged, and oxidized proteins [108–112]. Notably, the 20S proteasomes are more resistant to oxidative stress than 26S proteasomes and seem to be able to degrade oxidized proteins in an ATP-independent manner [113, 114]. Furthermore, i-proteasome expression is induced during oxidative stress in several inflammatory-based diseases in the CNS and in peripheral organs [30, 115, 116] and it provides enhanced cellular resistance to oxidative stress, at least in part by an increased degradation rate of oxidized proteins compared to s-proteasome [117]. Indeed, the blocked expression of  $\beta$ 1i subunit by siRNA significantly reduces the adaptive response to mild oxidative stress in mouse embryonic fibroblasts [116],  $\beta$ 5i-depleted retinal pigment epithelial cell viability is more compromised than wild type cells [30], and  $\beta$ 1i subunit  $-/-$  mice exhibit higher levels of protein carbonyls in brain and liver upon aging than those of their wild type littermates [118]. Accordingly, Seifert and coworkers [31] have shown an accumulation of oxidized and polyubiquitylated proteins and aggresome-like induced structures upon INF- $\gamma$  stimuli in the liver and brain of i-proteasome  $\beta$ 5i subunit  $-/-$  mice. Moreover,  $\beta$ 5i subunit deficient cells and tissues are not only more sensitive to apoptosis but also have a delayed activation of NF- $\kappa$ B after TNF- $\alpha$  stimulation [31]. This dependence of protein oxidation clearance on i-proteasome activity might be pivotal for MS because i-proteasome  $\beta$ 5i subunit  $-/-$  mice showed an earlier onset and worse clinical score than wild type mice in an EAE model [31] although this fact, recently, has been disputed by Nathan and colleagues [32].

Overall, these results suggest that i-proteasomes may influence onset and progression of MS by affecting the response of different cell types to the inflammatory aggression in the CNS.

## 6. Is Proteasome Inhibition a Potential Therapy of MS?

The administration of immunomodulatory drugs (glatiramer acetate and IFN- $\beta$ ) represents the first line therapy for RRMS, but these drugs are seldom useful towards the progressive form of MS [119]. The partial or total inefficacy of the common MS treatments in SPMS and PPMS patients demands the identification of novel therapies. The progressive forms of MS seem to be characterized by peculiar immunological mechanisms that differ from RRMS. In PPMS and SPMS the whole brain is affected and inflammation as well as axonal injury is diffuse, whereas in RRMS inflammation and tissue damage are more focalized in plaques [120]. In the progressive forms of MS, the CD4<sup>+</sup> and CD8<sup>+</sup> T cells and the B cells seem to be part of the pathological mechanisms, although with characteristics that differ from those observed in RRMS and without a clear correlation between immune cell activation and clinical measures of disease duration and severity, especially in PPMS [121]. Considering the complex pathogenic mechanisms at the basis of MS development, further studies would be needed to better characterize the role of different

immune system players, including proteasomes, autoAbs as well as specific Th17 and CD8<sup>+</sup> T cells, in the different forms of MS. These studies are likely to support the discovery of new diagnostic and prognostic biomarkers for different MS forms and to generate novel therapeutic drugs such as the specific proteasome inhibitors. Indeed, proteasome inhibitors have been utilised as therapeutic approach towards other diseases, such as multiple myeloma, and selective inhibitors for s- or i-proteasome have been recently developed [122].

Two factors could influence the success of novel therapies based on proteasome inhibitors: their toxicity profiles and their delivery pathways to the CNS and/or the periphery. Regarding the former, the experience of the first proteasome inhibitor, named Bortezomib, approved for clinical treatment of hematologic malignancies, showed that the toxicity could be a limiting factor [122]. However, this disadvantage can be controlled with new inhibitors specific for i-proteasome subunits that can therefore block proteasome activity only in specific cells or pathological conditions [122]. In such contest, the induction of i-proteasome expression in specific cell types upon MS onset—reviewed in Section 1—is a pivotal element ought to be borne in mind.

An additional critical issue is drug delivery. Indeed, the inhibition of i-proteasome is detrimental in tackling the oxidative stress during inflammation, leading to the accumulation of oxidised proteins [11]; this has been linked to the disputed observation that the depletion of  $\beta$ 5i subunit anticipated EAE onset [31, 32] (Table 1). However, further investigations have to be performed since the blockage of i-proteasome activity resulted in a decreased expression of inflammatory biomarkers in *ex vivo* analyses of microglia of a mouse model of Alzheimer's disease [19]. Conversely, an inhibition of i-proteasomes limited to the periphery and towards immune system components such as B and Th17 lymphocytes might be beneficial in treating MS. Noteworthy, the promising results of the clinical trials with the monoclonal antibody Rituximab for the treatment of MS (see Section 4) are consistent with the hypothesis that also a depletion of B cells might ameliorate MS disease. In mice, such depletion could be achieved by a defect in  $\beta$ 1i subunit expression [85] (Table 1).

Furthermore, Th17 cells could be targeted for ameliorating MS course. As i-proteasome inhibition decreases the activation of Th17 cells in mice [69, 70], it can be envisaged that i-proteasome inhibitors could be used to limit Th17 cell activation and EAE progression in mice (Table 1). The first test of this hypothesis could be obtained by treating EAE mice with inhibitors of the i-proteasome  $\beta$ 5i subunit, as it has been already done for other experimental disease models [69, 73]. Notably, a blockage of the i-proteasome activity along the Th17 cell pathway could be coupled to the therapeutic administration of probiotics (live beneficial bacteria) or prebiotics (compounds that stimulate the growth of beneficial bacteria) in EAE mice, given their common action on Th17 lymphocytes [67, 68]. Nonetheless, whether the modulation of gut microbiota could have similar beneficial effects also on MS is largely unknown. In EAE, the depletion or the strong modification of gut microbiota showed beneficial effects on the development of the disease [67, 68]. However, unlike

TABLE 1: Proteasome isoforms as potential targets of immunological pathways. The table summarizes the major results that may help future studies to understand how the inhibition of distinct proteasome isoforms may affect the development and progression of MS.

Final targets <sup>a</sup>	Anatomic area	Proteasome subunits inhibited <sup>b</sup>	MS forms	Potential effects <sup>c</sup>	References
CD8 <sup>+</sup> T cells	Thymus, lymph nodes, CNS	$\beta 1i, \beta 2i, \beta 5i$	NA	+/-	[22, 29, 38, 123, 124]
CD4 <sup>+</sup> Th17 cells	Thymus, lymph nodes, CNS, gut	$\beta 1i, \beta 2i, \beta 5i$	NA	+	[51, 67, 68, 70, 72, 73]
B cells	Thymus, lymph nodes, CNS	$\beta 1i$	RR, PP, SP	+	[80, 81, 85]
Proteasome Abs	Serum	NA	RR, PP, SP	NA	[90]
Circulating proteasome	Serum	NA	NA	NA	[94, 99]
CNS parenchyma	CNS	$\beta 5i$	NA	-	[31]

<sup>a</sup>Pathways that are directly or indirectly affected by treatment with proteasome inhibitors; <sup>b</sup>evidence from studies where the inhibition/depletion of specific proteasome subunit provided hints about their potential effect on MS; <sup>c</sup>these effects also include speculative arguments on how the proteasome subunit inhibition may affect specific pathways. The detrimental or beneficial effects are marked as “-” or “+,” respectively; NA = not available evidence.

mouse models, the human being has a broad variety of diet, environment, genetics, and early microbial exposure features that lead to highly diversified microbiota, which is furthermore extremely adaptable and variable over time [125, 126]. Therefore, the identification of a beneficial or detrimental microbiota towards MS might be strenuous.

While the potential inhibition of i-proteasome activity in B and Th17 cells points towards a beneficial effect against MS, the knowledge of the role of circulating proteasome and of proteasome Abs remains poor. Because of high levels of circulating proteasomes and proteasome Abs in the serum of MS patients [90, 99] a tempting speculation is that the production of proteasome Abs might aim to affect the circulating proteasome activity, although the role of circulating proteasomes in MS and more in general in the peripheral blood is largely unknown (Table 1). Further studies are mandatory to investigate such an issue because a therapy with proteasome inhibitors delivered through peripheral blood would immediately affect circulating proteasomes.

The potential effects of an i-proteasome inhibition within the CD8<sup>+</sup> T cell-mediated immune response are still unclear. This inhibition could affect therapy outcome depending on whether the drug is delivered in the periphery only or also in the CNS. Indeed, i-proteasome could influence the presentation of endogenously produced myelin antigens in oligodendrocytes (i.e., in CNS) and in bone marrow-derived APCs (i.e., in periphery) [123, 127], although the outcome of the activation of antimyelin CD8<sup>+</sup> T cells is still a matter of debate. For instance, in transgenic mice the induction of EAE by HLA-A\*03-restricted myelin epitope was hampered by the overexpression of HLA-A\*02 molecules, confirming the opposite (and interacting) action of MHC class I-restricted myelin epitopes on EAE onset [124]. Furthermore, it has been hypothesized that the expression of i-proteasome limits the generation of self-epitopes associated with autoimmune responses [38] and we have proposed that a link exists between a genetic protection toward MS and an i-proteasome polymorphism that impairs the generation of a specific MBP epitope [22]. We therefore conclude that i-proteasomes could play a role in the CD8<sup>+</sup> T cell-mediated immune response in MS, and further studies shall better define the role of CD8<sup>+</sup>

T cells in this pathology and identify which epitopes trigger a deleterious autoimmune CD8<sup>+</sup> T cell reaction and how they are generated by different proteasome isoforms.

## Abbreviations

APC:	Antigen presenting cells
AutoAbs:	Autoantibodies
BBB:	Blood-brain barrier
CNS:	Central nervous system
CSF:	Cerebrospinal fluid
DCs:	Dendritic cells
EAE:	Experimental autoimmune encephalitis
FoxP3:	Forkhead box P3
IFN:	Interferon
Ig:	Immunoglobulin
IL:	Interleukin
I-proteasome:	Immunoproteasome
MHC:	Major histocompatibility complex
MBP:	Myelin basic protein
MOG:	Myelin oligodendrocyte glycoprotein
MS:	Multiple sclerosis
PPMS:	Primary progressive MS
PCPS:	Proteasome-catalyzed peptide splicing
RRMS:	Relapsing-remitting MS
SPMS:	Secondary progressive phase MS
S-proteasome:	Standard proteasome
TcR:	T cell antigen receptor
Th:	T helper cells
TNF- $\alpha$ :	Tumor necrosis factor- $\alpha$
Treg:	Regulatory T cells
UPS:	Ubiquitin-proteasome system.

## Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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## References

- [1] E. M. Frohman, M. K. Racke, and C. S. Raine, "Multiple sclerosis. The plaque and its pathogenesis," *The New England Journal of Medicine*, vol. 354, no. 9, pp. 942–955, 2006.
- [2] P. K. Stys, G. W. Zamponi, J. van Minnen, and J. J. Geurts, "Will the real multiple sclerosis please stand up?" *Nature Reviews Neuroscience*, vol. 13, no. 7, pp. 507–514, 2012.
- [3] A. Compston and A. Coles, "Multiple sclerosis," *The Lancet*, vol. 372, no. 9648, pp. 1502–1517, 2008.
- [4] G. Giovannoni and G. Ebers, "Multiple sclerosis: the environment and causation," *Current Opinion in Neurology*, vol. 20, no. 3, pp. 261–268, 2007.
- [5] S. Jilek, M. Schluep, A. Harari et al., "HLA-B7-restricted EBV-specific CD8+ T cells are dysregulated in multiple sclerosis," *Journal of Immunology*, vol. 188, no. 9, pp. 4671–4680, 2012.
- [6] L. Fugger, M. A. Friese, and J. I. Bell, "From genes to function: the next challenge to understanding multiple sclerosis," *Nature Reviews Immunology*, vol. 9, no. 6, pp. 408–417, 2009.
- [7] B. D. Trapp and K.-A. Nave, "Multiple sclerosis: an immune or neurodegenerative disorder?" *Annual Review of Neuroscience*, vol. 31, pp. 247–269, 2008.
- [8] A. L. Goldberg, "Functions of the proteasome: from protein degradation and immune surveillance to cancer therapy," *Biochemical Society Transactions*, vol. 35, no. 1, pp. 12–17, 2007.
- [9] B. Dahlmann, "Role of proteasomes in disease," *BMC Biochemistry*, vol. 8, no. 1, article S3, 2007.
- [10] F. Schmidt, B. Dahlmann, H. K. Hustoft et al., "Quantitative proteome analysis of the 20S proteasome of apoptotic Jurkat T cells," *Amino Acids*, vol. 41, no. 2, pp. 351–361, 2011.
- [11] F. Ebstein, P.-M. Kloetzel, E. Krüger, and U. Seifert, "Emerging roles of immunoproteasomes beyond MHC class I antigen processing," *Cellular and Molecular Life Sciences*, vol. 69, no. 15, pp. 2543–2558, 2012.
- [12] A. M. Weissman, N. Shabek, and A. Ciechanover, "The predator becomes the prey: regulating the ubiquitin system by ubiquitylation and degradation," *Nature Reviews Molecular Cell Biology*, vol. 12, no. 9, pp. 605–620, 2011.
- [13] M. Bochtler, L. Ditzel, M. Groll, C. Hartmann, and R. Huber, "The proteasome," *Annual Review of Biophysics and Biomolecular Structure*, vol. 28, pp. 295–317, 1999.
- [14] D. Voges, P. Zwickl, and W. Baumeister, "The 26S proteasome: a molecular machine designed for controlled proteolysis," *Annual Review of Biochemistry*, vol. 68, pp. 1015–1068, 1999.
- [15] C. Pintado, M. P. Gavilán, E. Gavilán et al., "Lipopolysaccharide-induced neuroinflammation leads to the accumulation of ubiquitinated proteins and increases susceptibility to neurodegeneration induced by proteasome inhibition in rat hippocampus," *Journal of Neuroinflammation*, vol. 9, no. 1, article 87, 2012.
- [16] D. A. Ferrington and D. S. Gregerson, "Immunoproteasomes: structure, function, and antigen presentation," *Progress in Molecular Biology and Translational Science*, vol. 109, pp. 75–112, 2012.
- [17] N. Qureshi, D. C. Morrison, and J. Reis, "Proteasome protease mediated regulation of cytokine induction and inflammation," *Biochimica et Biophysica Acta*, vol. 1823, no. 11, pp. 2087–2093, 2012.
- [18] J. Zheng and O. A. Bizzozero, "Decreased activity of the 20S proteasome in the brain white matter and gray matter of patients with multiple sclerosis," *Journal of Neurochemistry*, vol. 117, no. 1, pp. 143–153, 2011.
- [19] M. Orre, W. Kamphuis, S. Dooves et al., "Reactive glia show increased immunoproteasome activity in Alzheimer's disease," *Brain*, vol. 136, no. 5, pp. 1415–1431, 2013.
- [20] S. Gohlke, M. Mishto, K. Textoris-Taube et al., "Molecular alterations in proteasomes of rat liver during aging result in altered proteolytic activities," *Age*, 2013.
- [21] C. Giannini, A. Kloss, S. Gohlke et al., "Poly-ub-substrate-degradative activity of 26S proteasome is not impaired in the aging rat brain," *PLoS ONE*, vol. 8, no. 5, Article ID e64042, 2013.
- [22] M. Mishto, E. Bellavista, C. Ligorio et al., "Immunoproteasome LMP2 60HH variant alters MBP epitope generation and reduces the risk to develop multiple sclerosis in Italian female population," *PLoS ONE*, vol. 5, no. 2, Article ID e9287, 2010.
- [23] J. Zheng, A. Dasgupta, and O. A. Bizzozero, "Changes in 20S subunit composition are largely responsible for altered proteasomal activities in experimental autoimmune encephalomyelitis," *Journal of Neurochemistry*, vol. 121, no. 3, pp. 486–494, 2012.
- [24] M. Mishto, C. Ligorio, E. Bellavista et al., "Immunoproteasome expression is induced in mesial temporal lobe epilepsy," *Biochemical and Biophysical Research Communications*, vol. 408, no. 1, pp. 65–70, 2011.
- [25] M. Kremer, A. Henn, C. Kolb et al., "Reduced immunoproteasome formation and accumulation of immunoproteasomal precursors in the brains of lymphocytic choriomeningitis virus-infected mice," *Journal of Immunology*, vol. 185, no. 9, pp. 5549–5560, 2010.
- [26] M. P. Gavilán, A. Castaño, M. Torres et al., "Age-related increase in the immunoproteasome content in rat hippocampus: molecular and functional aspects," *Journal of Neurochemistry*, vol. 108, no. 1, pp. 260–272, 2009.
- [27] M. Mishto, E. Bellavista, A. Santoro et al., "Immunoproteasome and LMP2 polymorphism in aged and Alzheimer's disease brains," *Neurobiology of Aging*, vol. 27, no. 1, pp. 54–66, 2006.
- [28] E. Bellavista, M. Mishto, A. Santoro, C. Bertoni-Freddari, R. B. Sessions, and C. Franceschi, "Immunoproteasome in Macaca fascicularis: no age-dependent modification of abundance and activity in the brain and insight into an in silico structural model," *Rejuvenation Research*, vol. 11, no. 1, pp. 73–82, 2008.
- [29] M. Groettrup, C. J. Kirk, and M. Basler, "Proteasomes in immune cells: more than peptide producers?" *Nature Reviews Immunology*, vol. 10, no. 1, pp. 73–78, 2010.
- [30] S. A. Hussong, R. J. Kapphahn, S. L. Phillips, M. Maldonado, and D. A. Ferrington, "Immunoproteasome deficiency alters retinal proteasome's response to stress," *Journal of Neurochemistry*, vol. 113, no. 6, pp. 1481–1490, 2010.
- [31] U. Seifert, L. P. Bialy, F. Ebstein et al., "Immunoproteasomes preserve protein homeostasis upon interferon-induced oxidative stress," *Cell*, vol. 142, no. 4, pp. 613–624, 2010.
- [32] J. A. Nathan, V. Spinnenhirn, G. Schmidtke, M. Basler, M. Groettrup, and A. L. Goldberg, "Immuno- and constitutive proteasomes do not differ in their abilities to degrade ubiquitinated proteins," *Cell*, vol. 152, no. 5, pp. 1184–1194.

- [33] M. A. Friese and L. Fugger, "Autoreactive CD8+ T cells in multiple sclerosis: a new target for therapy?" *Brain*, vol. 128, no. 8, pp. 1747–1763, 2005.
- [34] H. Yokote, S. Miyake, J. L. Croxford, S. Oki, H. Mizusawa, and T. Yamamura, "NKT cell-dependent amelioration of a mouse model of multiple sclerosis by altering gut flora," *The American Journal of Pathology*, vol. 173, no. 6, pp. 1714–1723, 2008.
- [35] T. Tsuchida, K. C. Parker, R. V. Turner, H. F. McFarland, J. E. Coligan, and W. E. Biddison, "Autoreactive CD8+ T-cell responses to human myelin protein-derived peptides," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 91, no. 23, pp. 10859–10863, 1994.
- [36] A. Jurewicz, W. E. Biddison, and J. P. Antel, "MHC class I-restricted lysis of human oligodendrocytes by myelin basic protein peptide-specific CD8 T lymphocytes," *Journal of Immunology*, vol. 160, no. 6, pp. 3056–3059, 1998.
- [37] Y. C. Q. Zang, S. Li, V. M. Rivera et al., "Increased CD8+ cytotoxic T cell responses to myelin basic protein in multiple sclerosis," *Journal of Immunology*, vol. 172, no. 8, pp. 5120–5127, 2004.
- [38] D. M. W. Zaiss, C. P. J. Bekker, A. Gröne, B. A. Lie, and A. J. A. M. Sijts, "Proteasome immunosubunits protect against the development of CD8 T cell-mediated autoimmune diseases," *Journal of Immunology*, vol. 187, no. 5, pp. 2302–2309, 2011.
- [39] J. D. Lünemann, N. Edwards, P. A. Muraro et al., "Increased frequency and broadened specificity of latent EBV nuclear antigen-1-specific T cells in multiple sclerosis," *Brain*, vol. 129, no. 6, pp. 1493–1506, 2006.
- [40] S. Jilek, M. Schlupe, P. Meylan et al., "Strong EBV-specific CD8+ T-cell response in patients with early multiple sclerosis," *Brain*, vol. 131, no. 7, pp. 1712–1721, 2008.
- [41] P. Höllsberg, H. J. Hansen, and S. Haahr, "Altered CD8+ T cell responses to selected Epstein-Barr virus immunodominant epitopes in patients with multiple sclerosis," *Clinical and Experimental Immunology*, vol. 132, no. 1, pp. 137–143, 2003.
- [42] S. Cepok, D. Zhou, R. Srivastava et al., "Identification of Epstein-Barr virus proteins as putative targets of the immune response in multiple sclerosis," *Journal of Clinical Investigation*, vol. 115, no. 5, pp. 1352–1360, 2005.
- [43] J. Ishizuka, K. Grebe, E. Shenderov et al., "Quantitating T cell cross-reactivity for unrelated peptide antigens," *Journal of Immunology*, vol. 183, no. 7, pp. 4337–4345, 2009.
- [44] N. Vigneron, V. Stroobant, J. Chapiro et al., "An antigenic peptide produced by peptide splicing in the proteasome," *Science*, vol. 304, no. 5670, pp. 587–590, 2004.
- [45] A. Dalet, N. Vigneron, V. Stroobant, K.-I. Hanada, and B. J. Van Den Eynde, "Splicing of distant peptide fragments occurs in the proteasome by transpeptidation and produces the spliced antigenic peptide derived from fibroblast growth factor-5," *Journal of Immunology*, vol. 184, no. 6, pp. 3016–3024, 2010.
- [46] J. Liepe, M. Mishto, K. Textoris-Taube et al., "The 20S proteasome splicing activity discovered by SpliceMet," *PLoS Computational Biology*, vol. 6, no. 6, Article ID e1000830, 2010.
- [47] M. Mishto, A. Goede, K. T. Taube et al., "Driving forces of proteasome-catalyzed peptide splicing in yeast and humans," *Molecular and Cellular Proteomics*, vol. 11, no. 10, pp. 1008–1023, 2012.
- [48] H.-G. Rammensee, "Protein surgery," *Nature*, vol. 427, no. 6971, pp. 203–204, 2004.
- [49] K. Mori, M. Itoi, N. Tsukamoto, H. Kubo, and T. Amagai, "The perivascular space as a path of hematopoietic progenitor cells and mature T cells between the blood circulation and the thymic parenchyma," *International Immunology*, vol. 19, no. 6, pp. 745–753, 2007.
- [50] E. Bettelli, Y. Carrier, W. Gao et al., "Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells," *Nature*, vol. 441, no. 7090, pp. 235–238, 2006.
- [51] M. S. Maddur, P. Miossec, S. V. Kaveri, and J. Bayry, "Th17 cells: biology, pathogenesis of autoimmune and inflammatory diseases, and therapeutic strategies," *The American Journal of Pathology*, vol. 181, no. 1, pp. 8–18, 2012.
- [52] J. S. Tzartos, M. A. Friese, M. J. Craner et al., "Interleukin-17 production in central nervous system-infiltrating T cells and glial cells is associated with active disease in multiple sclerosis," *The American Journal of Pathology*, vol. 172, no. 1, pp. 146–155, 2008.
- [53] T. Carlson, M. Kroenke, P. Rao, T. E. Lane, and B. Segal, "The Th17-ELR+ CXC chemokine pathway is essential for the development of central nervous system autoimmune disease," *Journal of Experimental Medicine*, vol. 205, no. 4, pp. 811–823, 2008.
- [54] V. Siffrin, H. Radbruch, R. Glumm et al., "In Vivo imaging of partially reversible Th17 cell-induced neuronal dysfunction in the course of encephalomyelitis," *Immunity*, vol. 33, no. 3, pp. 424–436, 2010.
- [55] Y. Komiyama, S. Nakae, T. Matsuki et al., "IL-17 plays an important role in the development of experimental autoimmune encephalomyelitis," *Journal of Immunology*, vol. 177, no. 1, pp. 566–573, 2006.
- [56] L. Cosmi, L. Maggi, V. Santarlasci et al., "Identification of a novel subset of human circulating memory CD4+ T cells that produce both IL-17A and IL-4," *Journal of Allergy and Clinical Immunology*, vol. 125, no. 1–3, pp. 222–30.e1–222–30.e4, 2010.
- [57] K. Berer and G. Krishnamoorthy, "Commensal gut flora and brain autoimmunity: a love or hate affair?" *Acta Neuropathologica*, vol. 123, no. 5, pp. 639–651, 2012.
- [58] J. Bienenstock and S. Collins, "99th Dahlem Conference on Infection, Inflammation and Chronic Inflammatory Disorders: psycho-neuroimmunology and the intestinal microbiota: clinical observations and basic mechanisms," *Clinical and Experimental Immunology*, vol. 160, no. 1, pp. 85–91, 2010.
- [59] S. M. Collins, M. Surette, and P. Bercik, "The interplay between the intestinal microbiota and the brain," *Nature Reviews Microbiology*, vol. 10, no. 11, pp. 735–742, 2012.
- [60] I. I. Ivanov, K. Atarashi, N. Manel et al., "Induction of intestinal Th17 cells by segmented filamentous bacteria," *Cell*, vol. 139, no. 3, pp. 485–498, 2009.
- [61] C. O'Mahony, P. Scully, D. O'Mahony et al., "Commensal-induced regulatory T cells mediate protection against pathogen-stimulated NF- $\kappa$ B activation," *PLoS Pathogens*, vol. 4, no. 8, Article ID e1000112, 2008.
- [62] K. Atarashi, T. Tanoue, T. Shima et al., "Induction of colonic regulatory T cells by indigenous Clostridium species," *Science*, vol. 331, no. 6015, pp. 337–341, 2011.
- [63] A. Jamshidian, V. Shaygannejad, A. Pourazar, S. H. Zarkesh-Esfahani, and M. Gharagozloo, "Biased Treg/Th17 balance away from regulatory toward inflammatory phenotype in relapsed multiple sclerosis and its correlation with severity of symptoms," *Journal of Neuroimmunology*, vol. 262, no. 1–2, pp. 106–112, 2013.
- [64] S. Lavasani, B. Dzhabazov, M. Nouri et al., "A novel probiotic mixture exerts a therapeutic effect on experimental autoimmune encephalomyelitis mediated by IL-10 producing

- regulatory T cells,” *PLoS ONE*, vol. 5, no. 2, Article ID e9009, 2010.
- [65] J. Ochoa-Repáraz, D. W. Mielcarz, L. E. Ditrio et al., “Role of gut commensal microflora in the development of experimental autoimmune encephalomyelitis,” *Journal of Immunology*, vol. 183, no. 10, pp. 6041–6050, 2009.
- [66] J. Ochoa-Repáraz, D. W. Mielcarz, Y. Wang et al., “A polysaccharide from the human commensal *Bacteroides fragilis* protects against CNS demyelinating disease,” *Mucosal Immunology*, vol. 3, no. 5, pp. 487–495, 2010.
- [67] Y. K. Lee, J. S. Menezes, Y. Umesaki, and S. K. Mazmanian, “Proinflammatory T-cell responses to gut microbiota promote experimental autoimmune encephalomyelitis,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 108, supplement 1, pp. 4615–4622, 2011.
- [68] K. Berer, M. Mues, M. Koutrolos et al., “Commensal microbiota and myelin autoantigen cooperate to trigger autoimmune demyelination,” *Nature*, vol. 479, no. 7374, pp. 538–541, 2011.
- [69] T. Muchamuel, M. Basler, M. A. Aujay et al., “A selective inhibitor of the immunoproteasome subunit LMP7 blocks cytokine production and attenuates progression of experimental arthritis,” *Nature Medicine*, vol. 15, no. 7, pp. 781–787, 2009.
- [70] K. W. Kalim, M. Basler, C. J. Kirk, and M. Groettrup, “Immunoproteasome subunit LMP7 deficiency and inhibition suppresses Th1 and Th17 but enhances regulatory T cell differentiation,” *Journal of Immunology*, vol. 189, no. 8, pp. 4182–4193, 2012.
- [71] W. Strober, I. Fuss, and P. Mannon, “The fundamental basis of inflammatory bowel disease,” *Journal of Clinical Investigation*, vol. 117, no. 3, pp. 514–521, 2007.
- [72] N. Schmidt, E. Gonzalez, A. Visekruna et al., “Targeting the proteasome: partial inhibition of the proteasome by bortezomib or deletion of the immunosubunit LMP7 attenuates experimental colitis,” *Gut*, vol. 59, no. 7, pp. 896–906, 2010.
- [73] M. Basler, M. Dajee, C. Moll, M. Groettrup, and C. J. Kirk, “Prevention of experimental colitis by a selective inhibitor of the immunoproteasome,” *Journal of Immunology*, vol. 185, no. 1, pp. 634–641, 2010.
- [74] S. Oh, C. Cudrici, T. Ito, and H. Rus, “B-cells and humoral immunity in multiple sclerosis. Implications for therapy,” *Immunologic Research*, vol. 40, no. 3, pp. 224–234, 2008.
- [75] P. Engel, J. A. Gómez-Puerta, M. Ramos-Casals, F. Lozano, and X. Bosch, “Therapeutic targeting of B cells for rheumatic autoimmune diseases,” *Pharmacological Reviews*, vol. 63, no. 1, pp. 127–156, 2011.
- [76] A. C. Chan, “B cell immunotherapy in autoimmunity: 2010 update,” *Molecular Immunology*, vol. 48, no. 11, pp. 1344–1347, 2011.
- [77] B. Serafini, B. Rosicarelli, R. Magliozzi, E. Stigliano, and F. Aloisi, “Detection of ectopic B-cell follicles with germinal centers in the meninges of patients with secondary progressive multiple sclerosis,” *Brain Pathology*, vol. 14, no. 2, pp. 164–174, 2004.
- [78] M. Duddy, M. Niino, F. Adatia et al., “Distinct effector cytokine profiles of memory and naive human B cell subsets and implication in multiple sclerosis,” *Journal of Immunology*, vol. 178, no. 10, pp. 6092–6099, 2007.
- [79] A. Bar-Or, L. Fawaz, B. Fan et al., “Abnormal B-cell cytokine responses a trigger of T-cell-mediated disease in MS?” *Annals of Neurology*, vol. 67, no. 4, pp. 452–461, 2010.
- [80] K. Hawker, P. O’Connor, M. S. Freedman et al., “Rituximab in patients with primary progressive multiple sclerosis: results of a randomized double-blind placebo-controlled multicenter trial,” *Annals of Neurology*, vol. 66, no. 4, pp. 460–471, 2009.
- [81] S. L. Hauser, E. Waubant, D. L. Arnold et al., “B-cell depletion with rituximab in relapsing-remitting multiple sclerosis,” *The New England Journal of Medicine*, vol. 358, no. 7, pp. 676–688, 2008.
- [82] N. L. Monson, P. D. Cravens, E. M. Frohman, K. Hawker, and M. K. Racke, “Effect of rituximab on the peripheral blood and cerebrospinal fluid B cells in patients with primary progressive multiple sclerosis,” *Archives of Neurology*, vol. 62, no. 2, pp. 258–264, 2005.
- [83] L. Kappos, D. Li, P. A. Calabresi et al., “Ocrelizumab in relapsing-remitting multiple sclerosis: a phase 2, randomised, placebo-controlled, multicentre trial,” *The Lancet*, vol. 378, no. 9805, pp. 1779–1787, 2011.
- [84] S. Lulu and E. Waubant, “Humoral-targeted immunotherapies in multiple sclerosis,” *Neurotherapeutics*, vol. 10, no. 1, pp. 34–43, 2013.
- [85] S. E. Hensley, D. Zanker, B. P. Dolan et al., “Unexpected role for the immunoproteasome subunit LMP2 in antiviral humoral and innate immune responses,” *Journal of Immunology*, vol. 184, no. 8, pp. 4115–4122, 2010.
- [86] M. Maldonado, R. J. Kapphahn, M. R. Terluk et al., “Immunoproteasome deficiency modifies the alternative pathway of NFkappaB signaling,” *PLoS ONE*, vol. 8, no. 2, Article ID e56187, 2013.
- [87] M. W. Lin, D. Suan, K. Lenton et al., “Differentiating patterns of oligoclonal banding in the cerebrospinal fluid improves diagnostic utility for multiple sclerosis,” *Pathology*, vol. 44, no. 3, pp. 248–250, 2012.
- [88] O. Stich, S. Jarius, B. Kleer, C. Rasiah, R. Voltz, and S. Rauer, “Specific antibody index in cerebrospinal fluid from patients with central and peripheral paraneoplastic neurological syndromes,” *Journal of Neuroimmunology*, vol. 183, no. 1-2, pp. 220–224, 2007.
- [89] M. K. Storch, S. Piddlesden, M. Haltia, M. Iivanainen, P. Morgan, and H. Lassmann, “Multiple sclerosis: in situ evidence for antibody- and complement-mediated demyelination,” *Annals of Neurology*, vol. 43, no. 4, pp. 465–471, 1998.
- [90] I. Mayo, J. Arribas, P. Villoslada et al., “The proteasome is a major autoantigen in multiple sclerosis,” *Brain*, vol. 125, no. 12, pp. 2658–2667, 2002.
- [91] H. Thuy-Tien, M. Haugen, J. Aarseth, A. Storstein, and C. A. Vedeler, “Proteasome antibodies in patients with cancer or multiple sclerosis,” *Scandinavian Journal of Immunology*, vol. 67, no. 4, pp. 400–403, 2008.
- [92] N. H. Beyer, J. Milthers, B. A.-M. Lauridsen, G. Houen, and L. J. Frederiksen, “Autoantibodies to the proteasome in monosymptomatic optic neuritis may predict progression to multiple sclerosis,” *Scandinavian Journal of Clinical and Laboratory Investigation*, vol. 67, no. 7, pp. 696–706, 2007.
- [93] M. Brychcy, U. Kuckelkorn, G. Hausdorf et al., “Anti-20S proteasome autoantibodies inhibit proteasome stimulation by proteasome activator PA28,” *Arthritis and Rheumatism*, vol. 54, no. 7, pp. 2175–2183, 2006.
- [94] S. U. Sixt and B. Dahlmann, “Extracellular, circulating proteasomes and ubiquitin: incidence and relevance,” *Biochimica et Biophysica Acta*, vol. 1782, no. 12, pp. 817–823, 2008.
- [95] C. Jakob, K. Egerer, P. Liebisch et al., “Circulating proteasome levels are an independent prognostic factor for survival in multiple myeloma,” *Blood*, vol. 109, no. 5, pp. 2100–2105, 2007.

- [96] O. Mueller, T. Anlasik, J. Wiedemann et al., "Circulating extracellular proteasome in the cerebrospinal fluid: a study on concentration and proteolytic activity," *Journal of Molecular Neuroscience*, vol. 46, no. 3, pp. 509–515, 2012.
- [97] R. C. Lai, S. S. Tan, B. J. Teh et al., "Proteolytic potential of the MSC exosome proteome: implications for an exosome-mediated delivery of therapeutic proteasome," *International Journal of Proteomics*, vol. 2012, Article ID 971907, 14 pages, 2012.
- [98] G. A. Roth, B. Moser, C. Krenn et al., "Heightened levels of circulating 20S proteasome in critically ill patients," *European Journal of Clinical Investigation*, vol. 35, no. 6, pp. 399–403, 2005.
- [99] A. Minagar, W. Ma, X. Zhang et al., "Plasma ubiquitin-proteasome system profile in patients with multiple sclerosis: correlation with clinical features, neuroimaging, and treatment with interferon-beta-1b," *Journal of Neurology Research*, vol. 34, no. 6, pp. 611–618, 2012.
- [100] C. Stadelmann, C. Wegner, and W. Brück, "Inflammation, demyelination, and degeneration: recent insights from MS pathology," *Biochimica et Biophysica Acta*, vol. 1812, no. 2, pp. 275–282, 2011.
- [101] R. E. Gonsette, "Neurodegeneration in multiple sclerosis: the role of oxidative stress and excitotoxicity," *Journal of the Neurological Sciences*, vol. 274, no. 1-2, pp. 48–53, 2008.
- [102] H. Lassmann and J. van Horsen, "The molecular basis of neurodegeneration in multiple sclerosis," *FEBS Letters*, vol. 585, no. 23, pp. 3715–3723, 2011.
- [103] L. Haider, M. T. Fischer, J. M. Frischer et al., "Oxidative damage in multiple sclerosis lesions," *Brain*, vol. 134, no. 7, pp. 1914–1924, 2011.
- [104] S. M. Smerjac and O. A. Bizzozero, "Cytoskeletal protein carbonylation and degradation in experimental autoimmune encephalomyelitis," *Journal of Neurochemistry*, vol. 105, no. 3, pp. 763–772, 2008.
- [105] J. Zheng and O. A. Bizzozero, "Accumulation of protein carbonyls within cerebellar astrocytes in murine experimental autoimmune encephalomyelitis," *Journal of Neuroscience Research*, vol. 88, no. 15, pp. 3376–3385, 2010.
- [106] L. Papa and P. Rockwell, "Persistent mitochondrial dysfunction and oxidative stress hinder neuronal cell recovery from reversible proteasome inhibition," *Apoptosis*, vol. 13, no. 4, pp. 588–599, 2008.
- [107] P. G. Sullivan, N. B. Dragicevic, J.-H. Deng et al., "Proteasome inhibition alters neural mitochondrial homeostasis and mitochondria turnover," *Journal of Biological Chemistry*, vol. 279, no. 20, pp. 20699–20707, 2004.
- [108] C. T. Aiken, R. M. Kaake, X. Wang, and L. Huang, "Oxidative stress-mediated regulation of proteasome complexes," *Molecular and Cellular Proteomics*, vol. 10, no. 5, Article ID R110.006924, 2011.
- [109] S. Grimm, A. Höhn, and T. Grune, "Oxidative protein damage and the proteasome," *Amino acids*, vol. 42, no. 1, pp. 23–38, 2012.
- [110] T. Jung and T. Grune, "The proteasome and its role in the degradation of oxidized proteins," *IUBMB Life*, vol. 60, no. 11, pp. 743–752, 2008.
- [111] T. Grune, K. Merker, G. Sandig, and K. J. A. Davies, "Selective degradation of oxidatively modified protein substrates by the proteasome," *Biochemical and Biophysical Research Communications*, vol. 305, no. 3, pp. 709–718, 2003.
- [112] L. Farout and B. Friguet, "Proteasome function in aging and oxidative stress: implications in protein maintenance failure," *Antioxidants and Redox Signaling*, vol. 8, no. 1-2, pp. 205–216, 2006.
- [113] T. Reinheckel, N. Sitte, O. Ullrich, U. Kuckelkorn, K. J. A. Davies, and T. Grune, "Comparative resistance of the 20 S and 26 S proteasome to oxidative stress," *Biochemical Journal*, vol. 335, no. 3, pp. 637–642, 1998.
- [114] M. Kästle and T. Grune, "Proteins bearing oxidation-induced carbonyl groups are not preferentially ubiquitinated," *Biochimie*, vol. 93, no. 6, pp. 1076–1079, 2011.
- [115] S. Kotamraju, S. Matalon, T. Matsunaga, T. Shang, J. M. Hickman-Davis, and B. Kalyanaraman, "Upregulation of immunoproteasomes by nitric oxide: potential antioxidative mechanism in endothelial cells," *Free Radical Biology and Medicine*, vol. 40, no. 6, pp. 1034–1044, 2006.
- [116] A. M. Pickering, A. L. Koop, C. Y. Teoh, G. Ermak, T. Grune, and K. J. A. Davies, "The immunoproteasome, the 20S proteasome and the PA28 $\alpha\beta$  proteasome regulator are oxidative-stress-adaptive proteolytic complexes," *Biochemical Journal*, vol. 432, no. 3, pp. 585–594, 2010.
- [117] A. M. Pickering and K. J. Davies, "Differential roles of proteasome and immunoproteasome regulators Pa28 $\alpha$ , Pa28 $\beta$ , Pa28 $\gamma$  and Pa200 in the degradation of oxidized proteins," *Archives of Biochemistry and Biophysics*, vol. 523, no. 2, pp. 181–190, 2012.
- [118] Q. Ding, S. Martin, E. Dimayuga, A. J. Bruce-Keller, and J. N. Keller, "LMP2 knock-out mice have reduced proteasome activities and increased levels of oxidatively damaged proteins," *Antioxidants and Redox Signaling*, vol. 8, no. 1-2, pp. 130–135, 2006.
- [119] D. Bates, "Treatment effects of immunomodulatory therapies at different stages of multiple sclerosis in short-term trials," *Neurology*, vol. 76, supplement 1, no. 1, pp. S14–S25, 2011.
- [120] C. F. Lucchinetti, J. Parisi, and W. Bruck, "The pathology of multiple sclerosis," *Neurologic Clinics*, vol. 23, no. 1, pp. 77–105, 2005.
- [121] G. Frisullo, D. Plantone, A. Marti et al., "Type 1 immune response in progressive multiple sclerosis," *Journal of Neuroimmunology*, vol. 249, no. 1-2, pp. 112–116, 2012.
- [122] E. Bellavista, F. Andreoli, M. Parenti et al., "Immunoproteasome in cancer and neuropathologies: a new therapeutic target?" *Current Pharmaceutical Design*, vol. 19, no. 4, pp. 702–718, 2013.
- [123] A. Perchellet, I. Stromnes, J. M. Pang, and J. Goverman, "CD8+ T cells maintain tolerance to myelin basic protein by 'epitope theft,'" *Nature Immunology*, vol. 5, no. 6, pp. 606–614, 2004.
- [124] M. A. Friese, K. B. Jakobsen, L. Friis et al., "Opposing effects of HLA class I molecules in tuning autoreactive CD8 + T cells in multiple sclerosis," *Nature Medicine*, vol. 14, no. 11, pp. 1227–1235, 2008.
- [125] Human Microbiome Project Consortium, "Structure, function and diversity of the healthy human microbiome," *Nature*, vol. 486, no. 7402, pp. 207–214, 2012.
- [126] M. Candela, E. Biagi, S. Maccaferri, S. Turroni, and P. Brigidi, "Intestinal microbiota is a plastic factor responding to environmental changes," *Trends in Microbiology*, vol. 20, no. 8, pp. 385–391, 2012.
- [127] A. Perchellet, T. Brabb, and J. M. Goverman, "Crosspresentation by nonhematopoietic and direct presentation by hematopoietic cells induce central tolerance to myelin basic protein," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 37, pp. 14040–14045, 2008.

## Review Article

# Immunotherapy of Neuromyelitis Optica

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Neuromyelitis optica (NMO) is a chronic inflammatory disease of the central nervous system that affects the optic nerves and spinal cord resulting in visual impairment and myelopathy. There is a growing body of evidence that immunotherapeutic agents targeting T and B cell functions, as well as active elimination of proinflammatory molecules from the peripheral blood circulation, can attenuate disease progression. In this review, we discuss the immunotherapeutic options and the treatment strategies in NMO. We also analyze the pathogenic mechanisms of the disease in order to provide recommendations regarding treatments.

## 1. Introduction

Neuromyelitis optica (NMO), also known as Devic's disease, is a chronic inflammatory disease of the central nervous system (CNS) that preferentially targets the optic nerves and spinal cord [1]. The overall disease incidence has been estimated at 1:100,000 and that it has a predilection for middle-aged, non-Caucasian females [2]. NMO spectrum disorders (NMOSD) encompass a variation of this classical picture in that patients may have brain involvement or a more limited presentation such as isolated transverse myelitis or an optic neuritis [3]. Historically, many thought of NMO as a rare variant of multiple sclerosis (MS). Given the identification of unique clinical and radiological differences and the discovery of the NMO-IgG, an autoantibody against aquaporin-4 (aqp4), it is now understood to be its own entity with distinct pathogenesis, diagnostic criteria, prognosis, and treatment [1–5].

Until recently, NMO was considered a disease of limited therapeutic options and poor prognosis. Research over the last decade brought new understanding of the disease pathogenesis that translated into immunotherapy directed against this disease. Moreover, there is a growing body of evidence that NMO can be controlled by immunotherapeutics targeting its cellular and humoral immune mechanisms. We review the immunotherapy of NMO, the various treatment options, and the clinical strategies that are typically encountered in practice.

## 2. Neuromyelitis Optica: An Overview

NMO is a neurological disorder that classically presents as a case of severe bilateral optic neuritis associated with a transverse myelitis [1–5]. The typical disease onset is either acute or subacute, and the symptoms are likely to persist without treatment. Optic neuritis results in decreased or a complete loss of vision. Transverse myelitis is usually extensive and spans more than 3 consecutive vertebral segments. Deficits related to myelitis include paralysis and sensory loss below the lesion level along with gait impairment. Additional complications may include phrenic nerve paralysis, loss of sphincter control, dysautonomia, and painful tonic spasms. Brainstem (medulla oblongata and area postrema) can be involved at times with resultant persistent nausea and hiccups [6].

Magnetic resonance imaging (MRI) is used for diagnosis and monitoring of the disease [1, 4]. Optic nerve and spinal cord lesions appear as hyperintense on T2- and hypointense on T1-weighted images and enhance with gadolinium when they are inflamed. In the acute phase, the inflamed lesions also enlarge in size secondary to tissue edema. Inflammation may persist for months and result in tissue atrophy [7]. MRI lesions involving the brainstem, hypothalamus, and periventricular white matter may be seen in typical NMOSD and sometimes late in the disease course of NMO [1–3]. Independent of imaging, visual evoked potentials and CSF studies can be helpful in establishing the diagnosis [4, 8]. Optic coherence tomography (OCT) may also be used to

monitor the extent and the degree of progression of optic neuropathy [9].

NMO follows a relapsing-remitting clinical course in 70–90% of all patients [2]. Such a clinical course is correlated with female gender, older age of disease onset, longer (>3 months) optic neuritis-myelitis interval, and presence of systemic autoimmunity [2, 3]. Seropositivity for anti-aqp4 antibody is also a strong predictor for future disease relapses [10]. A monophasic clinical course tends to occur in young males. Neurological disability in relapsing-remitting disease appears to be a cumulative result of disease relapses [2]. After five years, approximately 50% of affected individuals have significant visual or motor impairment and require assistive devices for ambulation [2]. This time frame of five years is also notable for a mortality rate of 32% with the relapsing-remitting disease and 10% with the monophasic disease. Most patients expire from disease complications such as respiratory failure, urosepsis, and pulmonary embolism [2–4].

The etiology of NMO is unknown but it is believed to be an autoimmune disorder triggered by an environmental factor, possibly an infection, in genetically susceptible individuals [11–13]. The principal effector in NMO is the self-reactive, complement-activating anti-aqp4 antibody [14]. Aqp4 is a transmembrane protein that regulates the flow of water in cells. It is expressed by CNS astrocytes and astrocytic processes surrounding small blood vessels at the glia limitans [15]. The autoantibody has the capacity to bind to aqp4 on the astrocytic foot processes and then recruit and activate complement. This leads to the mobilization of polymorphonuclear cells (neutrophils and eosinophils), inflammation, and tissue swelling [16, 17].

Recent studies indicate that Th17 cells (a T cell subset producing interleukin 17) specific to aqp4 may also be involved in the disease pathogenesis [18]. They are implicated in the breakdown of the blood-brain barrier allowing extravasation of anti-aqp4 antibody and complement, along with recruitment of polymorphonuclear cells to the lesion sites. Pathologically, NMO lesions involve both the white and gray matter. They contain perivascular deposits of immune complexes, activated complement, and inflammatory cellular infiltrates [19]. The cellular infiltrates are composed of mononuclear and polymorphonuclear cells. Astrocytes targeted by the autoimmune response display cytopathic changes and downregulate the expression of aqp4 in a vasculocentric pattern [20]. Vascular hyalinization, tissue necrosis, demyelination, and gliosis commonly accompany the inflammatory process [18–22].

### 3. Immunotherapy of NMO

Immunotherapy of NMO is based on the current understanding of its pathogenesis. As summarized above, lesion formation involves interplay between cellular and humoral immune responses. It appears that the autoimmune reaction arises in the periphery with the appearance of anti-aqp4 antibodies and Th17 cells; then it progresses in cascade-like fashion. There are several points of augmentation and diversification of the autoimmune reaction, including complement activation and release of interleukin 17, which have

proinflammatory and chemotactic effects. This contributes to the recruitment of mononuclear and polymorphonuclear cells to the sites of initial inflammation. As the inflammatory reaction unfolds, a number of nonspecific injurious mechanisms become involved including vascular damage, tissue swelling, oxidative stress, astrocyte injury, and secondary demyelination. These processes can be suppressed by using immunotherapeutic agents targeting T and B cells (immunosuppressant, cytotoxic, and biologic agents) or by actively removing the pro-inflammatory factors from the peripheral blood circulation (therapeutic plasma exchange) (Figure 1). These therapeutic approaches are nonspecific to the self-reactive cells or antibodies but affect the immune system globally. Inflammation in NMO is necrotizing in nature and cannot be reversed; it can only be prevented or minimized with effective treatment.

Immunotherapy of NMO is divided into two parts: rescue therapy of an acute disease relapse and disease-modifying therapy. The goal of rescue therapy is to suppress the acute inflammatory process in order to achieve functional recovery in patients. Early and effective rescue therapy is essential in minimizing the degree of permanent tissue damage and neurological disability. Corticosteroids and plasma exchange (PLEX) are the most commonly used therapeutic modalities in acute settings. Corticosteroids exert global immunosuppressive and anti-inflammatory effects, whereas PLEX removes antibodies, complement, and cytokines from the blood. The effects of both treatment modalities are rapid and can be appreciated within days of their initiation. Corticosteroids are administered intravenously. The usual treatment regimen is that of methylprednisolone 1000 mg daily for 5 days, followed by an extended oral prednisone taper starting at 60–100 mg per day [23]. If the disease is refractory to corticosteroids, PLEX therapy should be considered. PLEX can be beneficial to patients with acute NMO and is frequently recommended as a second line therapy in refractory cases [24, 25]. In practice, methylprednisolone is administered first and if there is no treatment response within three to four days, PLEX may be initiated. PLEX is administered every other day (1.5x plasma volume per each exchange) over the course of two weeks. In our clinical experience, most patients exhibited functional improvement after four to six PLEX treatments. The patient's response to initial rescue therapy may not be immediate and should be reevaluated within a few weeks after its completion. In cases of a poor response or an early disease relapse, one may consider repeating the corticosteroid/PLEX treatment or using cytotoxic agents such as cyclophosphamide. The latter is administered as several monthly infusions at 0.5–1 g/m<sup>2</sup> and can be beneficial in refractory cases, particularly in patients with concomitant systemic autoimmune diseases [26].

The goal of disease-modifying treatment is to maintain disease remission and prevent future relapses. It is important to keep in mind that the majority of NMO patients are likely to have a relapsing-remitting disease. As such, their neurological disability will be cumulative and related to the frequency and severity of their disease relapses [27]. As many as 60% of all patients are likely to develop a disease relapse

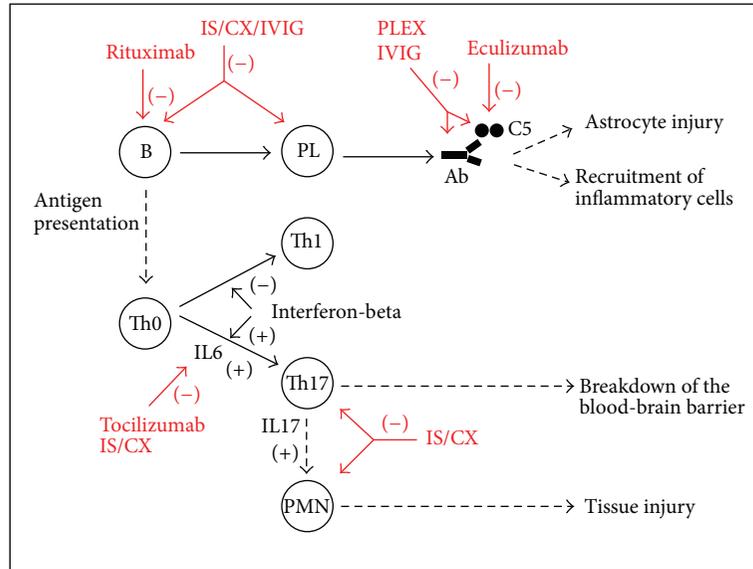


FIGURE 1: Summary of the mechanisms of action of immunotherapies in NMO. Abbreviations: B = B cell, C5 = protein 5 of complement, CX = cytotoxic agent, IL = interleukin, IS = immunosuppressant, IVIG = intravenous immunoglobulin, PL = plasma cell, PMN = polymorphonuclear cell, Th = T helper cell.

in the first year and 95% within three years of diagnosis [2]. In this respect early recognition of the predictors of relapsing-remitting disease is important. There are no randomized double blind placebo-controlled studies that have demonstrated the efficacy of any of the aforementioned treatment options. Most of the current knowledge is derived from anecdotal or small retrospective studies. Therefore, recommended treatments are based on the current understanding of disease pathogenesis, observed responses to treatment, tolerability, and the availability of resources.

Immunosuppressant agents interfering with the function of T and B cells have been shown to prevent disease relapses and reduce neurological disability in NMO. They can be viewed as steroid-sparing agents extending the beneficial effect of the rescue corticosteroid therapy. Azathioprine, perhaps the most commonly used oral immunosuppressant agent in NMO, primarily suppresses T cell function [23, 28]. The largest retrospective study involving 99 patients reported that azathioprine decreased the annualized relapse rate by 76% and either improved or stabilized disability in 40% of patients in a twelve-month period [28]. Azathioprine may be initiated at a dose of 50 mg daily or less and subsequently increased as tolerated to a target dose of 2-3 mg/kg/day (approximately 200–300 mg daily) either during or immediately after the intravenous methylprednisolone treatment. Doses lower than 2 mg/kg/day may have a limited effect on disease activity [28]. Prednisone in a prolonged tapering regimen from a dose of 100 mg down to 10 mg over a year may be added in order to compensate for the slow mechanism of action of azathioprine and to broaden the spectrum of immunosuppression. Once disease remission is achieved, then the medication can be continued as monotherapy for years at the lowest effective dose [23, 28].

Mycophenolate mofetil is another oral immunosuppressant that has the advantage of suppressing the proliferation of

both T and B cells, as well as the production of antibodies by plasma cells [29]. It is effective in patients with NMO at an average dose of 2000 mg daily and is generally well tolerated [29]. In a retrospective study involving 25 patients, treatment with mycophenolate mofetil was reported to decrease the annualized relapse rate in 71% of patients and improves disability in 91% of patients over a median follow-up of twenty-eight months [29]. Similar to azathioprine, adding corticosteroids (intravenous or oral) to mycophenolate mofetil treatment can potentiate its efficacy, particularly in the first several months of treatment. In addition, prednisone can prevent disease relapses in some cases as a sole disease-modifying agent. In these instances, doses at 25 mg or above every other day are necessary to maintain disease remission [30].

A few small case studies reported the disease-modifying effect of intermittent PLEX on NMO relapses [31, 32]. This approach may be used as a long-term extension of the rescue PLEX, especially in patients who have had a dramatic initial treatment response. It can be also considered as an alternative to immunosuppressants in the setting of treatment failure or significant side effects. While the frequency of intermittent PLEX sessions should be established empirically based on the duration of treatment-induced disease remissions, it is usually administered once every two to three months [31]. More frequent regimens on a weekly basis can be considered as well [32]. As previously noted, a small dose of daily prednisone of 5–20 mg daily can provide an add-on therapeutic benefit [31].

Rituximab is a monoclonal antibody against B cells (anti-CD 20), which can directly deplete this cell population from the peripheral blood circulation in a matter of a few weeks. This effect is global as B cells serve as precursors of antibody-producing plasma cells and are involved in the processes of antigen presentation and T cell activation. Rituximab has been reported to be effective, particularly in patients

who have failed oral immunosuppressant therapy [33–35]. The two largest retrospective studies, which included more than 20 patients, each reported significant reduction in the annualized relapse rate and improvement in neurological disability in more than 80%–90% of cases in nearly a two-year period [34, 35]. The drug can be administered intravenously at 375 mg/m once weekly for four weeks or at a flat dose of 1 g two weeks apart. Periodic retreatments are often necessary depending on the clinical response [36, 37]. Notably, the rituximab dose and frequency of administration can be tailored to the level of peripheral B cells, which should be maintained at zero.

Eculizumab is another monoclonal antibody that neutralizes complement protein 5 (anti-C5), thereby inhibiting the propagation of the complement cascade, the recruitment of inflammatory cells, and the formation of the membrane-attack complex. Recently, a small open-label study involving 14 NMO-IgG seropositive patients reported that biweekly intravenous administration of 900 mg of eculizumab (after a titration period) had a significant impact on the disease [38]. Twelve of 14 patients became relapse free after twelve months of treatment. Significant improvements in visual acuity and median disability scores were reported as well. None of the patients developed disease worsening. However, a return of disease activity was observed in 5 patients following discontinuation of the medication. Eculizumab administration was associated with significant suppression of serum complement activity and reduction of C5 levels in CSF, whereas medication discontinuation was associated with their normalization. NMO-IgG titers measured throughout the study remained unchanged.

Other authors reported benefit with other agents including cyclosporine, mitoxantrone, methotrexate, intravenous immunoglobulin (IVIG), and tocilizumab (anti-interleukin 6) [39–43]. For instance, intermittent administration of IVIG may be useful as an acute and chronic treatment in patients who have failed standard immunosuppressive therapy [42]. Tocilizumab blockade of interleukin 6, a cytokine that potentiates B cell survival and Th17 immune responses, may be effective in patients with highly active disease that are unresponsive to multiple immunosuppressive and cell depleting therapies [43]. Overall, these studies are retrospective, anecdotal, or small in size [39–43]. Nonetheless, NMO is a rare autoimmune disease that can be refractory to multiple treatments and every positive experience can be of potential value in clinical practice.

#### 4. Additional Considerations

Currently, there is no biomarker for therapeutic response in NMO. There are observations correlating effective immunotherapy to a decrease in anti-aqp4 antibody levels of patients [44]. However, there are no definitive studies establishing the significance of this autoantibody as a biomarker of treatment response. Moreover, it appears that seropositive and seronegative NMO patients do not differ in their responses to immunotherapy [45, 46]. Most of the treatment assessments are based on general neurologic or empirical principles. These include change in relapse rate or

neurological disability and appearance of new or gadolinium-enhancing lesions on MRI. In a few studies neurological improvement in patients treated with PLEX was reported to be associated with early treatment, rapid initial response, male gender, preserved leg reflexes, and absence of atrophy on MRI [46, 47]. In addition, there is evidence that preservation of retinal nerve fiber layer on OCT can be associated with a good treatment response to corticosteroids and PLEX [9, 32].

Certain laboratory tests reflecting the mechanism of action of medications may be useful in monitoring and predicting treatment responses in patients. For instance, a slight elevation of the erythrocyte mean corpuscular volume (MCV) more than 5 points above baseline following treatment with azathioprine may correlate with effective immune suppression and associated decline in patients' annualized relapse rate [28]. This increase in size of red blood cells is a metabolic effect of the medication and in fact some of its metabolites can be directly measured in these cells [48]. Elevation of MCV is well tolerated and is reversible with discontinuation of azathioprine [48]. Levels of mycophenolate mofetil metabolites such as mycophenolic acid and mycophenolic acid glucuronide can be directly measured in patient's blood [49]. Even though there are studies indicating the significance of therapeutic monitoring of mycophenolic acid in organ transplantation, its value in NMO remains to be established.

Rituximab is a cell depleting monoclonal antibody whose clinical benefit negatively correlates with levels of peripheral B cells [36, 50]. In some reported cases, rituximab failure was associated with rapid recovery of B cells after treatment. However, disease worsening on rituximab may have a more complex nature. Initial response to corticosteroids was identified as a negative predictor in some patients. This was hypothesized as being due to predominant T cell involvement with relatively less B cell involvement in the disease's pathogenesis [50]. Early disease worsening can be also due to extensive B cell death and secondary nonspecific activation of the immune system or by transient elevation of the anti-aqp4 antibody [50–52]. It is also important to mention that rituximab exerts little effect on certain CD20 negative B cells and on mature antibody-producing plasma cells, which may maintain the disease activity despite its presence.

Even though most of the immunotherapies are new to the NMO field, they are widely used in other autoimmune diseases and their side effects are well described in the medical literature. In general, NMO patients tolerate these therapies similarly to other patient populations. However, treatment of NMO patients with long-term immunosuppressants is complicated by their neurological disability and coexistent medical conditions. Therefore, systemic and organ-specific adverse effects should be expected and frequent monitoring is recommended. The most important adverse effects are myelosuppression and secondary leucopenias and lymphopenias. Significant myelosuppression associated with azathioprine use can occur in patients in whom the critical metabolizing enzyme thiopurine S-methyltransferase (TPMT) is either partially or completely inactivated [28, 53]. The latter can be seen in patients with homozygous or heterozygous mutations in the TPMT gene. These mutations may be found

in 10% of the population or associated with intake of enzyme inhibitors such as aspirin, allopurinol, and furosemide. Independently, myelosuppression can also be potentiated by carbamazepine, an anticonvulsant that is commonly used for neuropathic pain [54]. Finally, NMO patients are likely to be treated with multiple immunosuppressive and cytotoxic agents raising concerns about secondary malignancies as well as systemic or opportunistic infections [28, 29, 34, 38].

One should be aware that certain therapeutic agents that are commonly used in MS could actually worsen NMO. In particular, treatment with interferon-beta has been shown to increase disease activity in NMO, as well as to increase anti-aqp-4 antibody titers [55]. It is now recognized that disease mechanisms of MS and NMO involve different T cell subsets. Autoimmunity in MS is driven predominantly by Th1 cells (a T cell subset producing interferon-gamma), whose function is suppressed by interferon-beta. In contrast, NMO is a predominantly Th17-driven disease and administration of interferon-beta potentiates its pro-inflammatory effect on neutrophils and antibody production [56]. In addition, fingolimod and natalizumab may be associated with persisting or worsening NMO activity [57, 58]. Therefore, these medications should be avoided in patients suspected of having NMO. In clinical practice, NMO and NMOSD should be on the differential diagnosis in patients with suspected MS who worsen on interferon-beta, natalizumab, or fingolimod treatment. As a corollary, NMO patients with coexisting systemic autoimmune diseases should be carefully monitored for unintended treatment-induced disease worsening, as more than 30% of all NMO patients may have another autoimmune disease [59].

There is circumstantial evidence of an association between infections and NMO. Disease onset may be preceded by an infectious prodrome in up to 30% of all patients [2]. Chronic infections such as *Mycobacterium tuberculosis*, human immunodeficiency virus, *Helicobacter pylori*, and others may be present in patients with NMO [12, 60, 61]. One should consider investigating patients for chronic infections, particularly if environmental or personal risk factors for such infections can be identified. One may also consider the possibility of disease relapses being triggered by more ubiquitous microbial species. These can express immune epitopes with the capacity to cross-react and activate anti-aqp4 specific T and B cells [62–65]. The latter may be relevant to patients with chronic respiratory or bladder problems and decubitus ulcers, who are more prone for infectious complications. Clinical vigilance and at times prophylactic use of antibiotics and changes in immunotherapy may be warranted. In some NMO cases, specific antibiotic (antituberculosis) treatment has been reported to induce disease remission [66]. At this point, there is no available information regarding whether or not vaccination planning should be applied differently to NMO patients. Nonetheless, a clinician should consider a patients' general health status and their current immunosuppressive therapy.

Despite the fact that immunotherapy of NMO takes into account the relative predominance of certain humoral and cellular processes in the disease pathogenesis, it remains

nonspecific in nature and produces global immunosuppression. Recently, new experimental approaches directed against more disease-specific immune mechanisms were proposed [67]. Among these is the use of inhibitors of anti-aqp4 antibody binding. An example of this approach is aquaporin-4, a nonpathogenic monoclonal antibody against aqp4, which can function as a competitive inhibitor of the disease-associated NMO IgG. This strategy has generated promising results in some of the *in vitro* and *in vivo* models of NMO [68]. Another example is the treatment of patient sera with bacteria-derived endoglycosidase S. Such treatment causes IgG deglycosylation and converts the pathogenic anti-aqp4 antibodies into nonpathogenic target-blocking antibodies [69]. Development and implementation of disease-specific therapeutics may be an important step towards improving the treatment outcomes of the disease and solving some of the clinical dilemmas associated with chronic immunosuppression.

## 5. Conclusion

Clinical and basic science knowledge of NMO has dramatically increased over the last decade. Immunotherapy of NMO is still in its naissance but appears promising and certainly has changed the perception of NMO as an inevitably disabling or fatal disease. Perhaps, the most encouraging aspect is that a large number of treatment options may be used depending on the specific clinical settings. Issues that remain to be addressed include better and earlier recognition of patients with relapsing-remitting disease, identification of prognostic factors of treatment response, development of a biomarker of disease activity, and research on the infectious triggers of the disease. This is complicated by the fact that NMO is a rare disease such that the clinical experience with immunotherapies is still anecdotal. Multicenter, prospective, and controlled studies are required in order to identify the optimal immunotherapies for this disease.

## Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

## References

- [1] D. M. Wingerchuk, V. A. Lennon, S. J. Pittock, C. F. Lucchinetti, and B. G. Weinshenker, "Revised diagnostic criteria for neuromyelitis optica," *Neurology*, vol. 66, no. 10, pp. 1485–1489, 2006.
- [2] D. M. Wingerchuk, W. F. Hogancamp, P. C. O'Brien, and B. G. Weinshenker, "The clinical course of neuromyelitis optica (Devic's syndrome)," *Neurology*, vol. 53, no. 5, pp. 1107–1114, 1999.
- [3] D. M. Wingerchuk, V. A. Lennon, C. F. Lucchinetti, S. J. Pittock, and B. G. Weinshenker, "The spectrum of neuromyelitis optica," *The Lancet Neurology*, vol. 6, no. 9, pp. 805–815, 2007.
- [4] J. I. O'Riordan, H. L. Gallagher, A. J. Thompson et al., "Clinical, CSF, and MRI findings in Devic's neuromyelitis optica," *Journal of Neurology Neurosurgery and Psychiatry*, vol. 60, no. 4, pp. 382–387, 1996.

- [5] P. V. A. Lennon, D. M. Wingerchuk, T. J. Kryzer et al., "A serum autoantibody marker of neuromyelitis optica: distinction from multiple sclerosis," *The Lancet*, vol. 364, no. 9451, pp. 2106–2112, 2004.
- [6] T. Misu, K. Fujihara, I. Nakashima, S. Sato, and Y. Itoyama, "Intractable hiccup and nausea with periaqueductal lesions in neuromyelitis optica," *Neurology*, vol. 65, no. 9, pp. 1479–1482, 2005.
- [7] K. Tashiro, K. Ito, and Y. Maruo, "MR imaging of spinal cord in Devic disease," *Journal of Computer Assisted Tomography*, vol. 11, no. 3, pp. 516–517, 1987.
- [8] F. Masuhr, M. Busch, K. Wetzel, L. Harms, and E. Schielke, "Relapsing myelitis with pathological visual evoked potentials: a case of neuromyelitis optica?" *European Journal of Neurology*, vol. 9, no. 4, pp. 430–432, 2002.
- [9] D. B. Fernandes, A. S. Raza, R. G. Nogueira et al., "Evaluation of inner retinal layers in patients with multiple sclerosis or neuromyelitis optica using optical coherence tomography," *Ophthalmology*, vol. 57, no. 3, pp. 387–394, 2012.
- [10] B. G. Weinshenker, D. M. Wingerchuk, S. Vukusic et al., "Neuromyelitis optica IgG predicts relapse after longitudinally extensive transverse myelitis," *Annals of Neurology*, vol. 59, no. 3, pp. 566–569, 2006.
- [11] J. E. Cosnett, "Multiple sclerosis and neuromyelitis optica in tropical and subtropical countries," *Medical Hypotheses*, vol. 7, no. 1, pp. 61–63, 1981.
- [12] S. Yoshimura, N. Isobe, T. Matsushita et al., "Distinct genetic and infectious profiles in Japanese neuromyelitis optica patients according to anti-aquaporin 4 antibody status," *Journal of Neurology, Neurosurgery and Psychiatry*, vol. 84, no. 1, pp. 29–34, 2013.
- [13] H. Zéphir, I. Fajardy, O. Outteryck et al., "Is neuromyelitis optica associated with human leukocyte antigen?" *Multiple Sclerosis*, vol. 15, no. 5, pp. 571–579, 2009.
- [14] V. A. Lennon, T. J. Kryzer, S. J. Pittock, A. S. Verkman, and S. R. Hinson, "IgG marker of optic-spinal multiple sclerosis binds to the aquaporin-4 water channel," *Journal of Experimental Medicine*, vol. 202, no. 4, pp. 473–477, 2005.
- [15] P. Agre and D. Kozono, "Aquaporin water channels: molecular mechanisms for human diseases," *FEBS Letters*, vol. 555, no. 1, pp. 72–78, 2003.
- [16] L. Sabater, A. Giralt, A. Boronat et al., "Cytotoxic effect of neuromyelitis optica antibody (NMO-IgG) to astrocytes: an in vitro study," *Journal of Neuroimmunology*, vol. 215, no. 1-2, pp. 31–35, 2009.
- [17] T. Vincent, P. Saikali, R. Cayrol et al., "Functional consequences of neuromyelitis optica-IgG astrocyte interactions on blood-brain barrier permeability and granulocyte recruitment," *Journal of Immunology*, vol. 181, no. 8, pp. 5730–5737, 2008.
- [18] Y. Li, H. Wang, Y. Long, Z. Lu, and X. Hu, "Increased memory Th17 cells in patients with neuromyelitis optica and multiple sclerosis," *Journal of Neuroimmunology*, vol. 234, no. 1-2, pp. 155–160, 2011.
- [19] C. F. Lucchinetti, R. N. Mandler, D. McGavern et al., "A role for humoral mechanisms in the pathogenesis of Devic's neuromyelitis optica," *Brain*, vol. 125, no. 7, pp. 1450–1461, 2002.
- [20] S. F. Roemer, J. E. Parisi, V. A. Lennon et al., "Pattern-specific loss of aquaporin-4 immunoreactivity distinguishes neuromyelitis optica from multiple sclerosis," *Brain*, vol. 130, no. 5, pp. 1194–1205, 2007.
- [21] D. Lefkowitz and J. N. Angelo, "Neuromyelitis optica with unusual vascular changes," *Archives of Neurology*, vol. 41, no. 10, pp. 1103–1105, 1984.
- [22] R. N. Mandler, L. E. Davis, D. R. Jeffery, and M. Kornfeld, "Devic's neuromyelitis optica: a clinicopathological study of 8 patients," *Annals of Neurology*, vol. 34, no. 2, pp. 162–168, 1993.
- [23] R. N. Mandler, W. Ahmed, and J. E. Dencoff, "Devic's neuromyelitis optica: a prospective study of seven patients treated with prednisone and azathioprine," *Neurology*, vol. 51, no. 4, pp. 1219–1220, 1998.
- [24] B. G. Weinshenker, P. C. O'Brien, T. M. Petterson et al., "A randomized trial of plasma exchange in acute central nervous system inflammatory demyelinating disease," *Annals of Neurology*, vol. 46, no. 6, pp. 878–886, 1999.
- [25] M. Bonnan and P. Cabre, "Plasma exchange in severe attacks of neuromyelitis optica," *Multiple Sclerosis International*, vol. 2012, Article ID 787603, 9 pages, 2012.
- [26] A. Polgár, C. Rózsa, V. Müller, J. Matolcsi, G. Poór, and E. V. Kiss, "Devic's syndrome and SLE. Challenges in diagnosis and therapeutic possibilities based on two overlapping cases," *Autoimmunity Reviews*, vol. 10, no. 3, pp. 171–174, 2011.
- [27] D. M. Wingerchuk, S. J. Pittock, C. F. Lucchinetti, V. A. Lennon, and B. G. Weinshenker, "A secondary progressive clinical course is uncommon in neuromyelitis optica," *Neurology*, vol. 68, no. 8, pp. 603–605, 2007.
- [28] C. Costanzi, M. Matiello, C. F. Lucchinetti et al., "Azathioprine: tolerability, efficacy, and predictors of benefit in neuromyelitis optica," *Neurology*, vol. 77, no. 7, pp. 659–666, 2011.
- [29] A. Jacob, M. Matiello, B. G. Weinshenker et al., "Treatment of neuromyelitis optica with mycophenolate mofetil: retrospective analysis of 24 patients," *Archives of Neurology*, vol. 66, no. 9, pp. 1128–1133, 2009.
- [30] S. Watanabe, T. Misu, I. Miyazawa et al., "Low-dose corticosteroids reduce relapses in neuromyelitis optica: a retrospective analysis," *Multiple Sclerosis*, vol. 13, no. 8, pp. 968–974, 2007.
- [31] K. Miyamoto and S. Kusunoki, "Intermittent plasmapheresis prevents recurrence in neuromyelitis optica," *Therapeutic Apheresis and Dialysis*, vol. 13, no. 6, pp. 505–508, 2009.
- [32] B. O. Khatir, J. Kramer, M. Dukic, M. Palencia, and W. Verre, "Maintenance plasma exchange therapy for steroid-refractory neuromyelitis optica," *Journal of Clinical Apheresis*, vol. 27, no. 4, pp. 183–192, 2012.
- [33] B. A. C. Cree, S. Lamb, K. Morgan, A. Chen, E. Waubant, and C. Genain, "An open label study of the effects of rituximab in neuromyelitis optica," *Neurology*, vol. 64, no. 7, pp. 1270–1272, 2005.
- [34] A. Jacob, B. G. Weinshenker, I. Violich et al., "Treatment of neuromyelitis optica with rituximab: retrospective analysis of 25 patients," *Archives of Neurology*, vol. 65, no. 11, pp. 1443–1448, 2008.
- [35] G. S. Bedi, A. D. Brown, S. R. Delgado, N. Usmani, B. L. Lam, and W. A. Sheremata, "Impact of rituximab on relapse rate and disability in neuromyelitis optica," *Multiple Sclerosis*, vol. 17, no. 10, pp. 1225–1230, 2011.
- [36] S.-H. Kim, W. Kim, X. F. Li, I.-J. Jung, and H. J. Kim, "Repeated treatment with rituximab based on the assessment of peripheral circulating memory B cells in patients with relapsing neuromyelitis optica over 2 years," *Archives of Neurology*, vol. 68, no. 11, pp. 1412–1420, 2011.
- [37] H. L. Pellkofer, M. Krumbholz, A. Berthele et al., "Long-term follow-up of patients with neuromyelitis optica after repeated

- therapy with rituximab," *Neurology*, vol. 76, no. 15, pp. 1310–1315, 2011.
- [38] S. J. Pittock, V. A. Lennon, A. McKeon et al., "Eculizumab in AQP4-IgG-positive relapsing neuromyelitis optica spectrum disorders: an open-label study," *The Lancet Neurology*, vol. 12, no. 6, pp. 554–562, 2013.
- [39] T. Kageyama, M. Komori, K. Miyamoto et al., "Combination of cyclosporine A with corticosteroids is effective for the treatment of neuromyelitis optica," *Journal of Neurology*, vol. 260, no. 2, pp. 627–634, 2012.
- [40] B. Weinstock-Guttman, M. Ramanathan, N. Lincoff et al., "Study of mitoxantrone for the treatment of recurrent neuromyelitis optica (Devic disease)," *Archives of Neurology*, vol. 63, no. 7, pp. 957–963, 2006.
- [41] A. Minagar and W. Sheramata, "Treatment of Devic's with methotrexate and prednisone," *International Journal of Multiple Sclerosis Care*, vol. 2, pp. 39–43, 2000.
- [42] J. Bakker and L. Metz, "Devic's neuromyelitis optica treated with intravenous gamma globulin (IVIg)," *Canadian Journal of Neurological Sciences*, vol. 31, no. 2, pp. 265–267, 2004.
- [43] B. C. Kieseier, O. Stüve, T. Dehmel et al., "Disease amelioration with tocilizumab in a treatment-resistant patient with neuromyelitis optica: implication for cellular immune responses," *Archives of Neurology*, vol. 7, no. 3, pp. 390–393, 2012.
- [44] T. Takahashi, K. Fujihara, I. Nakashima et al., "Anti-aquaporin-4 antibody is involved in the pathogenesis of NMO: a study on antibody titre," *Brain*, vol. 130, no. 5, pp. 1235–1243, 2007.
- [45] M. Bonnan, R. Valentino, M. Bonnan, H. Mehdaoui, D. Smadja, and P. Cabre, "Plasma exchange in severe spinal attacks associated with neuromyelitis optica spectrum disorder," *Multiple Sclerosis*, vol. 15, no. 4, pp. 487–492, 2009.
- [46] Y. M. Lim, S. Y. Pyun, B. H. Kang, J. Kim, and K. K. Kim, "Factors associated with the effectiveness of plasma exchange for the recruitment of NMO-IgG positive neuromyelitis optica spectrum disorders," *Multiple Sclerosis Journal*, vol. 19, no. 9, pp. 1216–1218, 2012.
- [47] M. Keegan, A. A. Pineda, R. L. McClelland, C. H. Darby, M. Rodriguez, and B. G. Weinshenker, "Plasma exchange for severe attacks of CNS demyelination: predictors of response," *Neurology*, vol. 58, no. 1, pp. 143–148, 2002.
- [48] C. Cuffari, "A physician's guide to azathioprine metabolite testing," *Gastroenterology and Hepatology*, vol. 2, no. 1, pp. 58–63, 2006.
- [49] H. Jeong and B. Kaplan, "Therapeutic monitoring of mycophenolate mofetil," *Clinical Journal of the American Society of Nephrology*, vol. 2, no. 1, pp. 184–191, 2007.
- [50] B. M. Greenberg, D. Graves, G. Remington et al., "Rituximab dosing and monitoring strategies in neuromyelitis optica patients: creating strategies for therapeutic success," *Multiple Sclerosis Journal*, vol. 18, no. 7, pp. 1022–1026, 2012.
- [51] J. W. Lindsey, K. M. Meulmester, S. A. Brod, F. Nelson, and J. S. Wolinsky, "Variable results after rituximab in neuromyelitis optica," *Journal of the Neurological Sciences*, vol. 317, no. 1-2, pp. 103–105, 2012.
- [52] I. Nakashima, T. Takahashi, B. A. C. Cree et al., "Transient increases in anti-aquaporin-4 antibody titers following rituximab treatment in neuromyelitis optica, in association with elevated serum BAFF levels," *Journal of Clinical Neuroscience*, vol. 18, no. 7, pp. 997–998, 2011.
- [53] H. L. McLeod and C. Siva, "The thiopurine S-methyltransferase gene locus—implications for clinical pharmacogenomics," *Pharmacogenomics*, vol. 3, no. 1, pp. 89–98, 2002.
- [54] R. J. Flanagan and L. Dunk, "Haematological toxicity of drugs used in psychiatry," *Human Psychopharmacology*, vol. 23, no. 1, pp. 27–41, 2008.
- [55] J. Palace, M. I. Leite, A. Nairne, and A. Vincent, "Interferon beta treatment in neuromyelitis optica: increase in relapses and aquaporin 4 antibody titers," *Archives of Neurology*, vol. 67, no. 8, pp. 1016–1017, 2010.
- [56] R. C. Axtell, C. Raman, and L. Steinman, "Interferon- $\beta$  exacerbates Th17-mediated inflammatory disease," *Trends in Immunology*, vol. 32, no. 6, pp. 272–277, 2011.
- [57] J.-H. Min, B. J. Kim, and K. H. Lee, "Development of extensive brain lesions following fingolimod (FTY720) treatment in a patient with neuromyelitis optica spectrum disorder," *Multiple Sclerosis*, vol. 18, no. 1, pp. 113–115, 2012.
- [58] A. Jacob, M. Hutchinson, L. Elson et al., "Does natalizumab therapy worsen neuromyelitis optica?" *Neurology*, vol. 79, no. 10, pp. 1065–1066, 2012.
- [59] S. J. Pittock, V. A. Lennon, J. De Seze et al., "Neuromyelitis optica and non-organ-specific autoimmunity," *Archives of Neurology*, vol. 65, no. 1, pp. 78–83, 2008.
- [60] J. Sellner, B. Hemmer, and M. Mühlau, "The clinical spectrum and immunobiology of parainfectious neuromyelitis optica (Devic) syndromes," *Journal of Autoimmunity*, vol. 34, no. 4, pp. 371–379, 2010.
- [61] W. Li, M. Minohara, H. Piao et al., "Association of anti-Helicobacter pylori neutrophil-activating protein antibody response with anti-aquaporin-4 autoimmunity in Japanese patients with multiple sclerosis and neuromyelitis optica," *Multiple Sclerosis*, vol. 15, no. 12, pp. 1411–1421, 2009.
- [62] M. Koga, T. Takahashi, M. Kawai, K. Fujihara, and T. Kanda, "A serological analysis of viral and bacterial infections associated with neuromyelitis optica," *Journal of the Neurological Sciences*, vol. 300, no. 1-2, pp. 19–22, 2011.
- [63] Z. Ren, Y. Wang, T. Duan et al., "Cross-immunoreactivity between bacterial aquaporin-Z and human aquaporin-4: potential relevance to neuromyelitis optica," *Journal of Immunology*, vol. 189, no. 9, pp. 4602–4611, 2012.
- [64] P. O. Barros, U. C. Linhares, B. Teixeira et al., "High in vitro immune reactivity to *Escherichia coli* in neuromyelitis optica patients is correlated with both neurological disabilities and elevated plasma lipopolysaccharide levels," *Human Immunology*, vol. 74, no. 9, pp. 1080–1087, 2013.
- [65] M. Varrin-Doyer, C. M. Spencer, U. Schulze-Topphoff et al., "Aquaporin 4-specific T cells in neuromyelitis optica exhibit a Th17 bias and recognize Clostridium ABC transporter," *Annals of Neurology*, vol. 72, no. 1, pp. 53–64, 2012.
- [66] Y.-Q. Feng, N. Guo, F. Huang, X. Chen, Q.-S. Sun, and J.-X. Liu, "Anti-tuberculosis treatment for Devic's neuromyelitis optica," *Journal of Clinical Neuroscience*, vol. 17, no. 11, pp. 1372–1377, 2010.
- [67] M. C. Papadopoulos and A. S. Verkman, "Aquaporin 4 and neuromyelitis optica," *The Lancet Neurology*, vol. 11, no. 6, pp. 535–544, 2012.
- [68] L. Tradtrantip, H. Zhang, S. Saadoun et al., "Anti-aquaporin-4 monoclonal antibody blocker therapy for neuromyelitis optica," *Annals of Neurology*, vol. 71, no. 3, pp. 314–322, 2012.
- [69] L. Tradtrantip, J. Ratelade, H. Zhang, and A. S. Verkman, "Enzymatic deglycosylation converts pathogenic neuromyelitis optica anti-aquaporin-4 immunoglobulin G into therapeutic antibody," *Annals of Neurology*, vol. 73, no. 1, pp. 77–85, 2013.

## Review Article

# An Update in the Use of Antibodies to Treat Glioblastoma Multiforme

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Glioblastoma is a deadly brain disease and modest improvement in survival has been made. At initial diagnosis, treatment consists of maximum safe surgical resection, followed by temozolomide and chemoirradiation or adjuvant temozolomide alone. However, these treatments do not improve the prognosis and survival of patients. New treatment strategies are being sought according to the biology of tumors. The epidermal growth factor receptor has been considered as the hallmark in glioma tumors; thereby, some antibodies have been designed to bind to this receptor and block the downstream signaling pathways. Also, it is known that vascularization plays an important role in supplying new vessels to the tumor; therefore, new therapy has been guided to inhibit angiogenic growth factors in order to limit tumor growth. An innovative strategy in the treatment of glial tumors is the use of toxins produced by bacteria, which may be coupled to specific carrier-ligands and used for tumoral targeting. These carrier-ligands provide tumor-selective properties by the recognition of a cell-surface receptor on the tumor cells and promote their binding of the toxin-carrier complex prior to entry into the cell. Here, we reviewed some strategies to improve the management and treatment of glioblastoma and focused on the use of antibodies.

## 1. Introduction

Since the “magic bullet” concept proposed by Paul Ehrlich more than one century ago in which he describes that specific recognition and elimination of pathogen organisms or malignant cells by antibodies (Abs) is possible, many types of these molecules have been developed as tools against cancer. Abs have the capacity to travel through the blood, binding to specific tumor antigens on the surface of cells or recognizing other “tumor-related” targets, blocking ligand-receptor growth signals, some survival pathways, and finally eliciting tumor cell death [1].

Neuroepithelial tumors are the most common primary intracranial tumors of the central nervous system (CNS), and, unfortunately, malignant gliomas are the most lethal type of adult brain tumors. According to the World Health Organization (WHO), the classification of malignant gliomas is based on morphological similarities of the tumor cells

with nonneoplastic glial cells. Therefore, gliomas have been classified and graded on a malignant scale from I to IV as follows: astrocytic (grade I–IV), oligodendroglial (grade II–III), mixed oligoastrocytic (grade I–III), and ependymal tumors (grade I–II). Particularly, glioblastoma multiforme (GBM) is an anaplastic cellular, grade IV tumor with pleomorphic astrocytic cells with marked nuclear atypia and high mitotic rates [2]. Glioblastomas are rapidly evolving tumors typically with neoplastic infiltration of adjacent normal brain tissue and solid proliferating tumor at the periphery. Primary GBM arises de novo, whereas secondary GBM develops from preexisting low-grade astrocytomas [3]. Primary and secondary GBM are clinically indistinguishable. However, genotypically, there are some differences between them, which could be used in the search for improved treatment [3, 4]. Some of the genetic changes found in gliomas include amplification and/or overexpression of oncogenes, loss of tumor suppressor genes, DNA repairing genes through

mutation, loss of heterozygosity (LOH) in some chromosomes, or epigenetic mechanisms such as hypermethylation of promoters. These genetic changes result progressively in uncontrolled proliferation rates and loss of normal cell cycle control mechanisms, diminishing the ability of cells to undergo apoptosis in response to genotoxic agents, failure of DNA repairing mechanisms, increasing genetic instability, and deregulation of growth factor signaling pathways [5–7].

Glioblastoma tumors are heavily infiltrated by cells of myeloid origin, mainly microglia and macrophages [8]. These glioma-infiltrating myeloid cells (GIMs) comprise up to 30% of the total tumor mass and they have been implicated in several roles during GBM progression including proliferation, survival, motility, and immunosuppression. The origin of these GIMs seems to be from both resident brain macrophages (microglia) and newly recruited monocyte-derived macrophages from the circulation [9].

Despite the use of aggressive multimodality therapies that include surgery, radiotherapy, and chemotherapy, the median survival is only from 12 to 15 months. Additionally, the standard treatments for these tumors often result in debilitating motor and neurological deficits that alter physical skills and diminishing the quality of life of these patients. Nowadays, the literature describes the development of new strategies that could increase the prognostic and diminish the adverse events in patients. The known biology of glial tumors has allowed proposing some predictive markers that could be used to try a personalized treatment against gliomas. Between these markers is notable the role played by growth factors, such as the epidermal growth factor and the vascular epidermal growth factor, in gliomas progression and its treatment (Figure 1).

## 2. Role of Growth Factor Receptors in Tumorigenesis and Cancer Progression

The epidermal growth factor (EGF) has been implicated in supporting oncogenesis and progression of human solid tumors. EGF promotes tumor development amplifying the expression its tyrosine kinase epidermal growth factor receptor (EGFR) by increasing ligand-activated signaling through of its own receptor [31]. EGF plays a central role in cancer development since it is involved in crucial steps of tumor progression such as proliferation, angiogenesis, invasiveness, decreased apoptosis, and loss of cellular differentiation. In primary gliomas, the frequency of amplification of EGFR has been reported around in 40% of the examined cases [32].

Besides, several types of EGFR gene mutations have been reported in many tumors, including in GBM, and in nearly all cases these alterations have been related to EGFR amplification. Particularly, the mutant EGFR class III variant (so-called EGFRvIII) contains a deletion of 267 amino acids of the extracellular domain which creates a mutant with a unique extracellular domain [32]. This mutant EGFRvIII is ligand independent and it has been associated with constitutive activation of the wild receptor and failure to attenuate signaling by receptor downregulation. Also, it causes mitogenic effects, and it exhibits a more

powerful transforming activity [33, 34]. In this way, the constitutively active EGFRvIII can enhance cell proliferation in part by downregulation of p27 through activation of the phosphatidylinositol 3-kinase/serine-threonine kinase alpha (PI3K/Akt) pathway [35, 36].

Recent advances in targeted therapies have demonstrated that tyrosine kinase inhibitors (TKIs) have provided a marked benefit to subsets of patients whose tumors harbor specific genetic abnormalities. However, patients with EGFR mutations rapidly acquire resistance to TKI inhibitors decreasing the median time to disease progression to a few months [37].

Several strategies had been envisioned to overcome this resistance, such as dual-target inhibitors and multitarget and combined therapies. *In vitro* and *in vivo* properties and antitumor efficacy of the anti-EGFR/anti-CD3 bispecific monoclonal antibody (biMAb), so called M26.1, have been analyzed in previous reports. Treatment of IGROVI tumor-bearing mice with activated human lymphocytes coated with M26.1 F(ab')<sub>2</sub> significantly prolonged survival of the animals compared with tumor-bearing untreated mice. Therefore, these results strongly suggest the clinical usefulness of bispecific M26.1 F(ab')<sub>2</sub> as a targeting agent for local treatment of tumors such as glioma and ovarian cancers that express variable levels of EGFR [38].

Nowadays, some monoclonal antibodies (mAbs) have been developed that act or bind directly to EGFR mutated. Between these molecules, mAb-806 is a monoclonal anti-EGFRvIII antibody which significantly reduced the volume of tumors and increased in 61.5% the survival of mice-bearing xenografts of EGFRvIII gliomas compared to controls [39]. Patel and co-authors report that the mAb Cetuximab (c225), successfully targets and binds to U87MG cells expressing high levels of EGFRvIII leading to the internalization of the complex Cetuximab-EGFRvIII. A subsequent reduction was observed in the phosphorylated form of the mutant receptor in transfected cells and in a remarkable reduction (40–50%) in cell proliferation [40]. Y10 is another antibody specific for EGFRvIII whose intratumoral injection improved survival in animal models [41]. A range of potential therapies that target EGFR, or its constitutively active mutant EGFRvIII, are currently in development or in clinical trials for the treatment of GBM. Data from experimental studies evaluating these therapies have been very promising; however, their efficacy in the clinic has so far been limited by both upfront and acquired drug resistance in patients with recurrent high-grade gliomas [42].

## 3. Vascular Endothelial Growth Factor

Angiogenesis is the normal process by which new vessels are formed from preexisting vasculature. It is a physiological development that occurs in wound healing and when cells are exposed to hypoxia. Angiogenesis is driven by a wide variety of proangiogenic factors, mainly vascular endothelial growth factor (VEGF), and endogenous angiogenic inhibitor [43, 44]. VEGF consists of a family of 5 glycoproteins named VEGF-A, VEGF-B, VEGF-C, VEGF-D, and placental growth factor. They bind with their corresponding tyrosine kinase

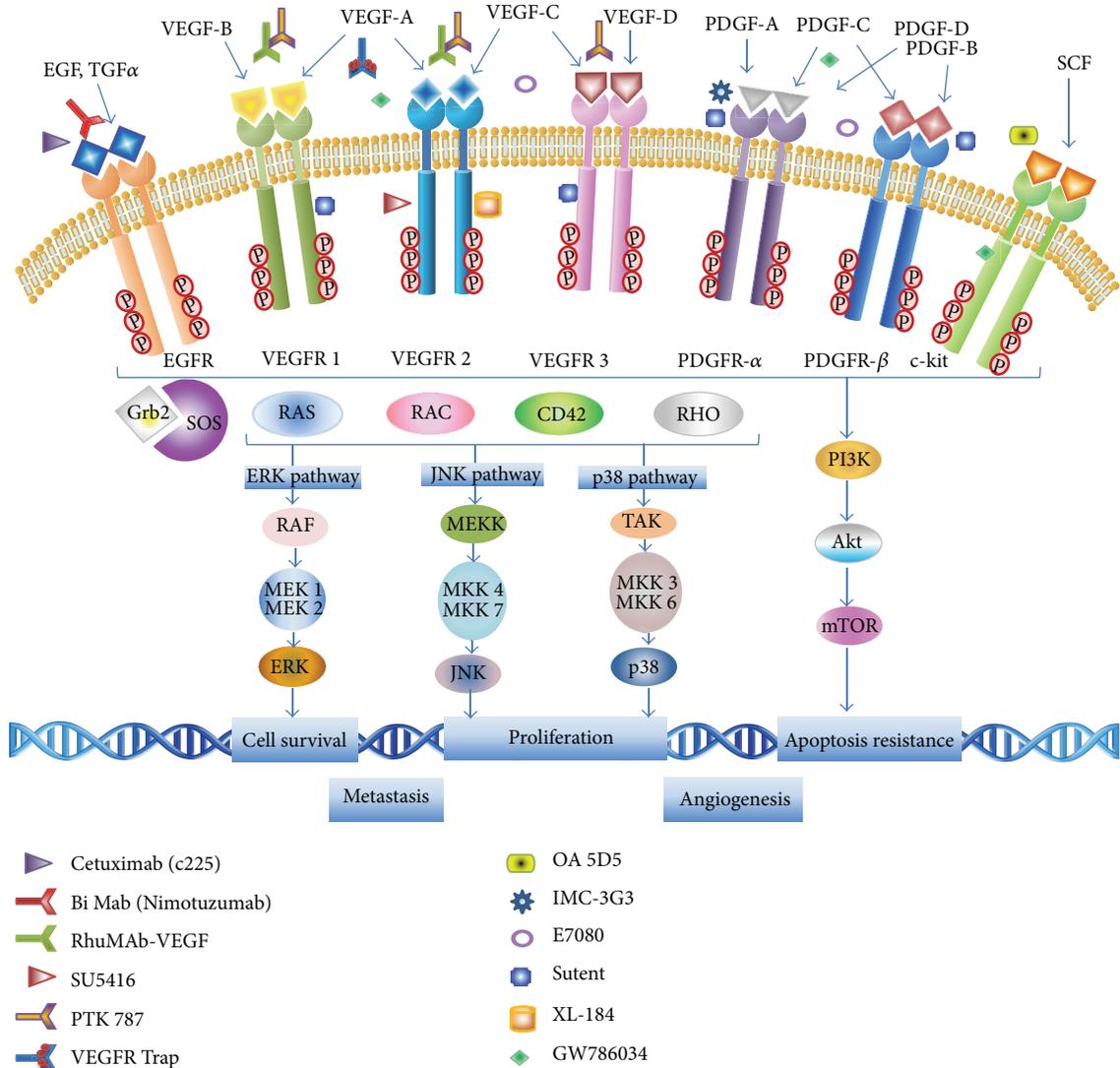


FIGURE 1: Antibodies used in gliomas treatment. Inhibition of tyrosine kinase downstream pathways signaling modulated by monoclonal antibodies to EGFR, VEGFR, PDGFR, and c-kit. Cdc42: cell division control protein 42, ERK: extracellular signal-regulated kinase, mTOR: mammalian target of rapamycin, PI3K: phosphatidylinositol 3-kinase, EGF(R): epidermal growth factor (receptor), Grb2: growth factor receptor-bound protein 2, JNK: c-Jun N-terminal kinase, MEK/MKK: mitogen-activated protein kinase kinases, PDGF(R): platelet derived growth factor (receptor), SOS: son of sevenless, TAK: TGFβ-activated kinase, TGF: transforming growth factor, and VEGF(R): vascular endothelial growth factor (receptor). Adapted and modified from Giamas et al. [30].

receptors (VEGFR-1, VEGFR-2, and VEGFR-3), activating a downstream signal, such as (PI3K), serin/trionine protein kinase alpha (Akt), and mitogen-activated protein kinase (MAPK), eliciting the development of angiogenesis and increasing vascular permeability, and the growth of lymphatic vessels that drain extravasated fluid, proteins, and tumor cells (lymphangiogenesis) [45].

In gliomas, it has been demonstrated that angiogenesis is an essential process that supplies oxygen and nutrients to developing tumors [46, 47]. The proangiogenic factors, mainly VEGF and endothelial, stromal, and tumoral cells, led to vessel growth and tumor expansion [48–50]. On base of these characteristics, some studies have been developed, with bevacizumab being the more tested.

Bevacizumab or RhuMAb-VEGF (Genentech) is a humanized monoclonal immunoglobulin G1 (IgG1) antibody against VEGF. Bevacizumab has a molecular weight of 149 kDa, and it selectively binds to all isoforms of human VEGF, therefore neutralizing VEGF's biologic activity through steric blockage of the binding of VEGF to its receptors VEGFR-1 and VEGFR-2 on the surface of endothelial cells [51]. In Phase I studies, bevacizumab has been safely administered alone and in combination with chemotherapy [52]. Besides, bevacizumab was associated with prolonged overall survival (OS) in phase III trials of metastatic colorectal [53] and non-small-cell lung [54] cancers and with prolonged progression-free survival (PFS) in metastatic breast [55] and renal cancers compared

with placebo or chemotherapy alone [56]. In patients with recurrent gliomas, the combination of bevacizumab with irinotecan (a cytotoxic prodrug which inhibits DNA replication and triggers apoptotic cell death) showed a safe toxicity, rate response over 63%, and increase of PFS until 23 weeks compared with other treatments [57]. Although bevacizumab improves survival and quality of life, an eventual tumor progression is observed. A better understanding of resistance mechanisms to VEGF inhibitors and identification of effective therapies after bevacizumab administration to avoid tumoral progression are currently a critical step for patients suffering glioblastoma. Validated biomarkers are strongly needed for predicting which patients are more likely to benefit and for monitoring response. Additionally, the Amgen Dana-Farber Cancer Institute has started a clinical trial to determine the efficacy of AMG386 plus bevacizumab in patients with recurrent glioblastoma (Clinical Trial NCT01290263). Recently, two randomized, double-blind, placebo-controlled studies designed to evaluate first-line use of bevacizumab added to the standard of care (chemoradiation (CRT) with temozolomide) in glioblastoma. The data shown did not improve the median overall survival. Additionally, patients were stratified based on MGMT promoter methylation and a 9-gene signature; however, they did not identify a group of patients who demonstrated benefit from first-line use of bevacizumab, but patients with MGMT promoter methylation and a favorable 9-gene signature showed a strong trend towards a worse outcome. Adverse events were higher for patients who received bevacizumab as first-line therapy with respect to hypertension, deep vein thrombosis/pulmonary embolism, wound issues, gastrointestinal perforations, and significant hemorrhagic events, and around 30% were discontinued after end study. The lack in response due to that the chronic use of bevacizumab changes glioblastomas from highly vascular tumors to nonvascular ones, which often do not respond to bevacizumab in most patients [58]. Although bevacizumab has shown some beneficial outcomes in a subgroup of patients, studies regarding the biology involved in the gliomagenesis and angiogenesis are necessary.

It is known that VEGF/VEGFR signaling can be inhibited at the level of the receptor or via downstream signaling pathways. Since VEGFR uses many of the same signaling pathways as epidermal growth factor receptors (EGFR), the above-mentioned mutant of this receptor EGFRvIII and the platelet-derived growth factor receptor (PDGFR), including the PI3K/Akt and Ras-MAPK pathways [59], many of their inhibitors may also target VEGFR-mediated signaling. At the receptor level, two VEGFR inhibitors, PTK787 (Novartis) and SU5416 (Semaxanib; Sugen/Pharmacia) are currently being evaluated and have been included in North American Brain Tumor Consortium- (NABTC-) sponsored clinical trials [60]. PTK787 inhibits all three VEGF receptors (VEGFR2-KDR/Flk-1; VEGFR1-FLT-1; and VEGFR3-FLT-4) and reduces the number of tumor microvessels in an animal model [61]. Currently, it is being evaluated in GBM patients in a Phase I clinical trial [62]. The inhibitor SU5416 also targets VEGFR2, and it has demonstrated impressive results

in animal models of a variety of cancers including GBM [63–65].

Aflibercept (VEGF Trap) is a recombinant fusion protein of the extracellular domains of VEGF fused to the Fc portion of IgG1, which binds with high affinity to both VEGF and PlGF. Preclinical studies in glioma animal models have demonstrated the efficacy of aflibercept to simultaneously inhibit angiogenesis and tumor invasion [66]. A recent study sponsored by the North American Brain Tumor Consortium Phase II of this recombinant protein demonstrated minimal activity in recurrent GBM [67]. However, preclinical data support a potential synergistic benefit of radiation therapy combined with aflibercept, and future studies may include combinations of this agent with radiation or chemotherapy [61]. Recently, Paz and Zhu correlate changes in cytokine and angiogenic factors as potential markers of toxicity to aflibercept [68]. They found that changes in IL-13 from baseline to 24 hrs predicted toxicities and increases in IL-1b, IL-6, and IL-10 at 24 hrs which were significantly associated with fatigue. The progression-free survival was 14.9 months for patients in the all-toxicity group and 9.0 months for patients in the on-target toxicity group compared to 4.3 months for those who did not develop any grade of toxicity. Authors conclude that profiling of IL-13 as a surrogate for endothelial dysfunction could individualize patients at risk during anti-angiogenic therapy, and identify those at higher risk for fatigue using IL-6 and IL-10 as markers [68].

Other mAb targeting the VEGFR-2 is Ramucirumab, which is a fully human monoclonal currently under development. Ramucirumab blocks VEGF binding and thwarting the angiogenic process. It is thought that inhibiting VEGFR-2 might yield superior outcomes in several solid tumors. Ramucirumab has demonstrated activity *in vitro* and in murine models against leukemia and ovarian cancer cell lines and in Phase I and II clinical trials against breast and gastric cancers [69].

Ramucirumab inhibits VEGFR-2 expression from normal endothelial cells, as well as tumor endothelial cells, impairing endothelial healing and hypercoagulability. Preliminary data suggest that ramucirumab is well tolerated, with manageable adverse effects. The safety of ramucirumab has not been reported on extensively; therefore, results from the many ongoing studies should shed light on this important area. VEGF inhibition increases the risk of bleeding events, as seen with bevacizumab (Avastin), another mAb that inhibits VEGF expression. Hypertension and renal toxicities are also not unexpected with ramucirumab. Based on safety data from trials of bevacizumab, investigators decided to exclude certain patient populations from subsequent trials of ramucirumab. These include patients who have brain metastases and a recent history of thrombotic events, non-healing wounds/ulcers, and major blood vessel encasement or invasion [7].

Currently, there is an interventional open-label study sponsored by the National Cancer Institute and ImClone LLC, where investigators plan to enroll 80 patients with brain and central nervous system tumors, particularly recurrent glioblastoma multiforme. One group will receive ramucirumab intravenously administered; the other group will

receive anti-PDGFR $\alpha$  monoclonal antibody IMC-3G3. Both treatments will be continued until disease progression or unacceptable toxicity. The primary outcome measure for this trial is progression-free survival at 6 months. Secondary outcome measures include objective tumor response rate, overall survival, acute and late toxicities, pharmacokinetic and pharmacodynamic profiles, and immunogenicity (ClinicalTrials.gov Identifier number NCT00895180).

Vandetanib (ZD6474) is an oral inhibitor that targets VEGFR, RET tyrosine kinase receptor family inhibitor, and the EGF receptor [70]. Treatment with vandetanib in a BT4C rat glioma model significantly altered the protein expression pattern in malignant glioma and normal brain [71, 72]. Following completion of a Phase I study of vandetanib, radiotherapy, and temozolomide in patients with newly diagnosed GBM, it was concluded that this inhibitor can be safely combined with radiotherapy. A Phase II study in which patients were randomized to receive vandetanib (100 mg) daily with radiotherapy and temozolomide or radiotherapy and temozolomide alone is currently underway [71].

Vatalanib (PTK787, ZK222584, or PTK/ZK) is an orally active, small-molecule VEGF R-TKi that inhibits all known VEGFRs, as well as PDGFR- $\beta$  and c-KIT, but is most selective for VEGFR-2. A Phase I pharmacokinetic study of vatalanib plus imatinib (a tyrosine kinase inhibitor which prevents phosphorylation and the subsequent activation of growth receptors) and hydroxyurea in recurrent malignant glioma patients determined that vatalanib at doses of up to 1000 mg twice a day combined with imatinib and hydroxyurea was well tolerated and may enhance antiangiogenesis activity [3]. Similar tolerance of this agent was found in a Phase I trial with biomarker studies of vatalanib in patients with newly diagnosed GBM treated with enzyme-inducing antiepileptic drugs and standard radiation and temozolomide [4]. An EORTC Phase I/II study on concomitant and adjuvant temozolomide and radiotherapy with vatalanib in newly diagnosed GBM reported that once-daily administration of up to 1000 mg of vatalanib in conjunction with concomitant temozolomide and radiotherapy was feasible and safe. However, a planned randomized Phase II trial was aborted owing to industry decision to halt further development of this agent [73].

As previously assessed, VEGFR and EGFR play a significant role in glioblastoma angiogenesis and proliferation, making tyrosine kinase (TK) receptors logical targets for treatment. Particularly, AEE788 is a novel reversible TK inhibitor of the EGF and VEGF receptors [8, 9, 46]. Recently, Reardon et al. evaluated the role of this TK inhibitor in sixty-four recurrent glioblastoma patients. Patients in group A experienced DLTs (proteinuria and stomatitis) at 550 mg; thereby 550 mg of AEE788 was the highest dose evaluated and dose limiting. Patients in group B received 800 mg of AEE788 and experienced diarrhea. The initially recommended dose for dose-expansion phase for Group A was 400 mg; additional patients received 250 mg to assess the hepatotoxicity. Most frequently reported adverse events (AEs) included diarrhea and rash. Serious AEs, most commonly grade 3/4 liver function test elevations, were responsible for treatment discontinuation in 17% of patients. AEE788 concentrations

were reduced by EIACD. The best overall response was stable disease (17%). Continuous, once-daily AEE788 was associated with unacceptable toxicity and minimal activity for the treatment of recurrent glioblastoma. The Phase I/II study of AEE788 in patients with recurrent/relapse glioblastoma was, therefore, discontinued prematurely [47].

Cediranib is an orally available pan-VEGFR tyrosine kinase inhibitor with a half-life of 22 hours compatible with once daily dosing [44] which has a subnanomolar 50% inhibitory concentration for VEGF receptors with additional activity against platelet-derived growth factor  $\beta$  and c-Kit. In a preliminary study on a subset of patients with recurrent glioblastoma, it was observed that cediranib treatment normalizes tumor vasculature and alleviates edema [74]. Recently, the final clinical efficacy, toxicity, and biomarker data on the entire cohort of patients treated on the first Phase II study of Cediranib in GBM was investigated, and authors report that Cediranib monotherapy for recurrent glioblastoma is associated with encouraging proportions of radiographic response, 6-month progression-free survival, and a steroid-sparing effect with manageable toxicity. They identified early changes in circulating molecules as potential biomarkers of response to cediranib [75]. The efficacy of this tyrosine kinase inhibitor in combination with lomustine chemotherapy in recurrent glioblastoma is now under clinical trials Phase III to compare the use of lomustine with cediranib, cediranib alone, or lomustine with placebo to see whether the combination or cediranib alone will be more effective than the chemotherapy alone (lomustine) in preventing the growth of cancer cells.

In addition to those mentioned inhibitors, pazopanib (GW786034) is another oral agent that inhibits the tyrosine kinases associated with the VEGF, PDGF, and KIT receptors. A Phase II study has evaluated the efficacy and safety of pazopanib in recurrent GBM patients at first or second relapse and no prior anti-VEGF/VEGFR therapy. Pazopanib was administered at a dose of 800 mg daily on 4-week cycles without planned interruptions. Pazopanib was reasonably well tolerated with manageable toxicities similar to other anti-VEGF/VEGFR agents. However, efficacy was absent without meaningful prolongation of PFS. The median PFS was 12 weeks (95% CI: 8–14 weeks), and only one patient had a PFS greater than or equal to 6 months. Thirty patients (86%) had died, and median survival was 35 weeks (95% CI: 24–47 weeks). However, *in situ* biological activity was suggested by the observation of radiographic responses in some patients [49].

It has been reported that increased mitogenic signaling and angiogenesis, frequently facilitated by somatic activation of EGF receptor (EGFR; ErbB1) and/or loss of PTEN, and VEGF overexpression, respectively, drive malignant glioma growth. Recently, it was suggested that patients with recurrent glioblastoma would exhibit differential antitumor benefit based on tumor PTEN/EGFRvIII status when treated with the antiangiogenic agent pazopanib and the ErbB inhibitor lapatinib. It was found that the six-month progression-free survival (PFS) rates in Phase II patients ( $n = 41$ ) were 0% and 15% in the PTEN/EGFRvIII-positive and PTEN/EGFRvIII-negative cohorts, respectively, leading to early finish of the

trial. Two patients (5%) had a partial response and 14 patients (34%) had stable disease lasting 8 or more weeks. In Phase I ( $n = 34$ ), the maximum tolerated regimen was not reached. On the basis of pharmacokinetic and safety review, a regimen of pazopanib (600 mg) plus lapatinib (1,000 mg), each twice daily, was considered safe. Concomitant EIACs reduced exposure to pazopanib and lapatinib. However, the antitumor activity of this combination at Phase II dose tested was limited. Pharmacokinetic data indicated that exposure to lapatinib was subtherapeutic in Phase II evaluation. Evaluation of intratumoral drug delivery and activity may be essential for hypothesis-testing trials with targeted agents in malignant gliomas [70]. Particularly, on 2007 was initiated a Phase II trial sponsored by the National Cancer Institute (USA) to determine the side effects and how well pazopanib works in treating patients with recurrent glioblastoma which has been completed the last February in 2013.

XL-184 (BMS-907351) is another pan-tyrosine kinase inhibitor, currently under development by Exelixis Inc. and Bristol-Myers Squibb Co., for the potential oral treatment of medullary thyroid cancer, glioblastoma multiforme, and non-small-cell lung cancer (NSCLC). The principal targets of XL-184 are the receptors to tyrosine kinase MET, RET, and VEGFR-2, but also it is reported that this drug displays its inhibitory activity against KIT, FLT3, and TEK. Preclinical studies demonstrated that XL-184 potently inhibited multiple receptor tyrosine kinases in several cancer cell lines and in animal xenograft models and that the drug exhibited significant oral bioavailability and blood-brain barrier penetration. A phase I clinical trial in patients with advanced solid malignancies indicated that XL-184 was accumulated dose-dependent way in the plasma, and it had a long terminal half-life. A Phase II trial in patients with progressive or recurrent glioblastoma (clinical trial number NCT00704288) revealed modest but promising median progression-free survival. Toxicity and side effects for the drug have generally been of low-to-moderate severity [35].

Another small-molecule tyrosine kinase inhibitor is Sunitinib malate (Sutent, SU11248), an orally active inhibitor that targets several receptors including c-KIT, VEGFR-1-3, PDGFR- $\alpha$ , PDGFR- $\beta$ , the class III receptor tyrosine kinase Flt3, colony stimulating factor-1R, and RET. A Phase I study of sunitinib and irinotecan for patients with recurrent malignant glioma demonstrated that the maximum tolerated dose of sunitinib was 50 mg administered once a day for 4 consecutive weeks followed by a 2-week rest combined with irinotecan (75 mg/m<sup>2</sup>) administered intravenously for an additional week. Reported dose-limiting toxicities were primarily hematological, and nonhematological toxicities included mucositis and dehydration. However, the PFS at 6 months was 24% and only one patient out of 25 achieved a radiographic response. Further development of a regimen using the dosing schedules for the combination of sunitinib and irinotecan was subsequently suspended owing to lack of efficacy [76, 77].

Another kinase inhibitor is E7080, whose targets include VEGFR, fibroblast growth factor receptor (FGFR), and PDGFR [68]. It has been shown that E7080 inhibits tumor

angiogenesis by targeting endothelial cells. A number of the targets of E7080 are also expressed on tumor cells showing direct effects on tumor cell behavior [78]. Using a panel of human tumor cell lines, the effect of E7080 on cell proliferation, migration, and invasion was determined, measuring the inhibition of FGFR and PDGFR signaling in the cells. Authors found that E7080 had little effect on tumor cell proliferation. However, it blocked migration and invasion at concentrations that inhibited FGFR and PDGFR signaling. Knockdown of PDGFR-b in U2OS osteosarcoma cells also inhibited cell migration, which could not be further inhibited in the presence of E7080. Furthermore, E7080 could not inhibit the migration of a PDGFR negative cell line. Therefore, E7080 does not significantly affect tumor cell proliferation, but it can inhibit their migration and invasion at concentrations that both inhibit its known targets and are achievable clinically. An interventional, multicenter, Phase II study is now under development in subjects with recurrent malignant glioma [52].

On the other hand, a large body of evidence suggests that the platelet-derived growth factor (PDGF) family and associated receptors are potential targets in oncology therapeutic development because of their critical roles in the proliferation and survival of some cancers and in the regulation and growth of the tumor stroma and blood vessels. Several small molecules that nonspecifically target the PDGF signaling axis are in current use or development as anticancer therapies [51, 66, 79]. However, for the majority of these agents, PDGF and its receptors are neither the primary targets nor the principal mediators of anticancer activity. IMC-3G3, a fully human monoclonal antibody of the immunoglobulin G subclass 1, specifically binds to the human PDGF receptor  $\alpha$  (PDGFR $\alpha$ ) with high affinity and blocks PDGF ligand binding and PDGFR $\alpha$  activation. The results of preclinical studies and the frequent expression of PDGFR $\alpha$  in many types of cancer and in cancer-associated stroma support a rationale for the clinical development of IMC-3G3 [67]. Currently, IMC-3G3 is being evaluated in Phase II clinical trials for patients with several types of solid malignancies, particularly glioblastoma multiforme, in order to determine how well IMC-3G3 monoclonal antibody works in GBM patients [61].

Sorafenib (Nexavar, BAY 43-9006) is a multitargeted small molecule that inhibits VEGFR-2, Flt3, PDGF receptor (PDGFR), FGF receptor-1, RAF, and c-KIT. It has been tested that sorafenib exerts antiglioma activity *in vitro* and *in vivo*. The treatment of established or patient-derived GBM cells with low concentrations of this inhibitor has been shown to cause a dose-dependent inhibition of proliferation, induction of apoptosis, and autophagy. Systemic delivery was well tolerated with intracranial glioma growth being suppressed via inhibition of cell proliferation and induction of apoptosis and autophagy, thus causing reduction of angiogenesis [57]. The inhibition of signal transducer and activator of transcription 3 (STAT3) by sorafenib has also been found to contribute to growth arrest and induction of apoptosis in GBM cells [80]. The efficacy of sorafenib with standard radiotherapy and temozolomide in the first-line treatment of patients with GBM was tested in patients with newly diagnosed GBM who

received concurrent radiotherapy (2.0 Gy per day; total dose 60 Gy) and temozolomide (at a dose of 75 mg/m<sup>2</sup> orally on days 1–5 every 28 days) and sorafenib (at a dose of 400 mg orally twice daily). The median PFS for the entire group was 6 months (95% CI: 3.7–7 months), with a 1-year PFS rate of 16%. The median OS was 12 months (95% CI: 7.2–16 months). The outcome of this trial yielded survival data similar to what has been reported with radiotherapy and temozolomide alone, suggesting that sorafenib has minimal activity against GBM when it is incorporated into initial management [81].

#### 4. Hepatocyte Growth Factor

The multifunctional growth factor scatter factor/hepatocyte growth factor (SF/HGF) and its receptor, c-Met, are important mediators of brain tumor growth and angiogenesis [82–84]. Until now, the well-known biological consequences of c-Met activation are invasion, cellular morphogenesis, motility, metastasis, immortalization, and angiogenesis. The effect achieved by tyrosine kinase inhibitors of multiple factors and pathways involved in tumor angiogenesis has demonstrated clinical benefit in some neoplasms, including glial tumors. The overexpressions of HGF and c-Met in a very high percentage of patients with solid tumors are associated with a poor outcome and could benefit from Met-targeted therapies. The response to hypoxia increases HGF release and c-Met signaling, and also enhances metastasis in untreated tumors; besides it might play an important role in the resistance to VEGF-targeted agents in cancer therapy [85]. The c-met receptor tyrosine kinase is encoded by the c-met protooncogene, and it has been widely implicated in tumor progression and invasion [86]. Both SF/HGF and c-Met are overexpressed in human glioblastomas, and these expression levels correlate with glioma malignancy grade and vascularity [87–90]. Even when overexpression of SF/HGF and/or c-Met promotes glioma growth and angiogenesis *in vivo* [91], targeting of SF/HGF with single monoclonal antibodies was found to be ineffective, and they were only effective when three antibodies were combined, suggesting that single antibodies against SF/HGF could not fully block the SF/HGF:c-Met binding [92]. Recently, a one-armed (OA) variant of the anti-c-Met antibody 5D5 [93] was developed at Genentech, which acts as a pure antagonist and it can inhibit the growth of cells dependent on SF/HGF:c-Met autocrine and paracrine signaling. Martens and coauthors developed a monovalent OA-5D5 antibody which successfully inhibited glioma growth in an orthotopic *in vivo* model [94].

#### 5. Cytotoxic Antibodies Drugs against Cancer Cells

Immunotoxins are a class of antineoplastic agents comprising a modified toxin linked to a cell-selective agent, such as a growth factor or antibody, for specifically targeting cancer cells [95]. The toxin may be any poison produced by an organism, including the bacterial toxins that cause tetanus, diphtheria, and so forth, or plants and animal toxins, such as ricin and snake venom [96]. A variety of toxins, mainly

from plants, fungi, or bacteria, have been characterized, structurally optimized for *in vitro* stability, activity, and safety, and evaluated in animal studies and clinical trials. These toxins generally consist of several domains: the cell-binding or cell-recognition domain, the translocation domain, which enables the release of the toxin into the cytosol, and the activity domain responsible for cytotoxicity. During the development of immunotoxins, the binding domain of these toxins is replaced by cancer-cell-specific ligands, which lead the modified toxins directly to their internalization via receptor-mediated endocytosis. Upon internalization, the catalytic domain of the toxin is cleaved in the late endosome, and it is translocated to the cytosol leading to cell death by various mechanisms [97].

The development of an immunotoxin involves the chemical coupling or genetic fusion of a cell-selective ligand with a complete toxin or a modified form of the toxin. Since most cytotoxic drugs have a low molecular weight (<1000 g/mol), they rapidly diffuse into tumor cells and healthy tissue. This leads to the known adverse effects, which appear either rapidly or emerge later as delayed toxicity. These unwanted side effects limit the use of potent drugs even if they achieve objective responses and seem to be beneficial for the patient. In an attempt to improve the efficacy of cytotoxic agents without raising the burden of side effects, researchers have devised strategies to prevent easy diffusion by binding the toxic drugs to macromolecules, such as antibodies, serum proteins, lectins, peptides, growth factors, and synthetic polymers [98] (Table 1).

Recombinant DNA techniques have been applied in the production of the last generation of immunotoxins to promote tumor specificity delivery, penetration, and to reduce the cost and complexity of production. The cell-binding domain of the toxin is genetically removed, and the modified toxin is fused with a ligand or with DNA elements encoding the Fv portion of an antibody in these constructs [99, 100]. The light- and heavy-chain variable fragments are either genetically linked (scFv) or held together by a disulfide bond (dsFv) [101].

Diphtheria toxin (DT) has a cell-binding domain at the C terminus (amino acids 482–539) and the A chain with ADP-ribosylation activity at the N terminus. The A chain catalyzes the transfer of adenosine diphosphate-(ADP-) ribose to EF-2, preventing the translocation of peptidyl-t-RNA on ribosomes, thereby blocking the protein synthesis and subsequently killing the cell [102–104]. A natural ligand for DT on the cell membrane is the heparin-binding epidermal growth factor-(EGF-) like precursor [105]. Recombinant DT is made by replacing the C terminal cell-binding domain with a ligand that binds to a growth factor receptor or the Fv fragment of an antibody. Variable truncation of the binding segments resulting in 389 and 486 amino acid length toxin conjugates has resulted in the formation of toxins DAB389 and DAB486, respectively [106]. Another modification of DT involves substitution of two amino acids in the B chain resulting in a new molecule cross-reacting material-107 (CRM-107) [107]. This modification reduces the nonspecific binding of DT to human cells by 8000 fold, thus increasing the toxin's tumor specificity to 10,000 fold. Unfortunately, a

TABLE 1: Classification of clinically used toxins based on their mechanism of action.

Toxins	Source	Classification of toxins			References
		Mechanism	Structure	Modifications	
ADP ribosylating toxins					
Diphtheria toxin	<i>Corynebacterium diphtheria</i>	ADP ribosylation of EF2	Activity (A chain), translocation (T), and binding (B) domains	(a) DT486 (b) DT388 or DT389 (deletion of cell-binding domain) (c) CRM107 point (mutation in cell-binding domain of DT)	[10–13]
<i>Pseudomonas</i> exotoxin	<i>Pseudomonas aeruginosa</i>	ADP ribosylation of EF2	Binding (Ia), translocation (II and Ib), and activity domains (III)	(a) PE40 and PE40KDEL (b) PE38 and PE38KDEL (c) PE38QQR (d) PE35	[10, 13–15]
Pore-forming toxins					
Cholera toxin	<i>Vibrio cholera</i>	ADP ribosylation of Gs, a subunit of G protein	Activity (A chain) and cell-binding domains (pentameric B chain)	CET40 (domains II and III)	[13, 16, 17]
Ribosome inactivating toxins					
Holotoxins-ricin	<i>Ricinus communis</i>	N-glycosylation of 28S rRNA	Activity and binding domains	(a) Ricin (b) Ricin A chain (RTA) (c) bR (blocked ricin) (d) dgA (deglycosylated ricin A chain)	[13, 18]
Hemitoxins-saporin (SAP), pokeweed antiviral protein (PAP)	<i>Saponaria officinalis</i> , <i>Phytolacca americana</i>	N-glycosylation of 28S rRNA	Single-chain proteins without binding domain		[13, 19]
Ribonucleases					
Fungal toxins-a-sarcin, restrictocin	<i>Aspergillus</i> sp.	Cleavage of 28S rRNA	Single-chain proteins without binding domain		[13, 19]
HPR, ECP, EDN	Human	Degradation of RNA	Single-chain proteins		[13, 20]

Some immunotoxins are presented which have been used as toxin-based therapeutic approaches in the treatment of several malignancies acting on different intracellular targets. ADP: adenosine diphosphate; EF2: elongation factor 2 during protein synthesis on the ribosome; DT: diphtheria toxin; DT388 or DT389: truncated forms of DT without the receptor-binding activity; CRM107: cross-reacting material-mutant of DT without the receptor binding; PE: *Pseudomonas* exotoxin A; PE40 and PE38: truncated forms of PE without the receptor-binding domain Ia; CET40: cholera exotoxin A; RTA: ricin toxin A; HPR: human pancreatic ribonuclease A; ECP: eosinophilic cationic protein; EDN: eosinophil-derived neurotoxin.

Phase III trial comparing Tf-CRM107 with the current gold standard treatment determined that it was ineffective, and further development was terminated [108].

*Pseudomonas aeruginosa* exotoxin A is a single peptide with three functional domains: domain Ia is the N terminal and cell-binding domain; domain II has the translocation activity; and domain III is the C terminal and it catalyzes the adenosine diphosphate (ADP) ribosylation that inactivates EF-2, which further blocks protein synthesis and causes cell death. The genetic excision of domain I results in a molecule termed PE 40 which retains its translocation function and EF-2 inhibition properties but is unable to kill human cells [109, 110]. Furthermore, removal of the domain should in turn decrease the hepatotoxicity of PE immunotoxins that is due to residual binding of domain to the hepatocyte. A genetically engineered PE molecule (so-called PE38KDEL) has amino acids 253–364 linked to amino acids 381–608 with a change in the carboxyl end of PE (KDEL) to increase

cytotoxic activity [111, 112]. PE38KDEL has been fused with a targeting moiety such as the antibody Fv portion, a growth factor, or cytokine. It was observed a much higher affinity for binding to cancer cell lines than the native PE immunotoxin, and it was very toxic to malignant cells [113, 114]. A Phase I trial of an immunotoxin made with an antibody attached to domains II and III of *Pseudomonas* exotoxin and EGFRvIII resulted in the formation of a new, tumor-specific extracellular sequence. Mice were immunized with a synthetic peptide corresponding to this sequence, and positive EGFRvIII cells were purified. After, they developed an immunotoxin by fusing the scFv sequences coding for domains II and III of *Pseudomonas* exotoxin A. The immunotoxin was very cytotoxic to cells expressing EGFRvIII. The combination of high affinity, cytotoxic activity, and stability makes this immunotoxin a strong candidate for further preclinical evaluation [115] (Table 2).

TABLE 2: Immunotoxins against gliomas.

Immunotoxin	Toxin used	Target antigen	Administrative route	Clinical trial phase	Number and type of tumor	Outcome	Adverse effect	References
IL-4(38-37)-PE38KDEL	(38-37) PE38KDEL	IL-4R	Intratumoral (CED)	I/II	31 (25 GBM and 6 AA)	Median survival 8.2 months; six-month survival was 52%	Headache, seizure, weakness, dysphasia, and hydrocephalus	[21–23]
IL13-PE38QQR	PE38QQR	IL-13R	Intratumoral (CED)	I/II/III	Phase II, 51 (46 GBM, 3 AA, other 2); Phase III, 296 recurrent GBM	Infusion MTIC was 0.5 $\mu\text{g}/\text{mL}$ ; up to 6 d well tolerated; median survival 42.7 weeks (95% CI, 35.6–55.6) for GBM in Phase II and 36.4 weeks in Phase III, comparable to Gliadel Wafer	Headache, dysphasia, seizure, weakness, and pulmonary embolism	[24–26]
TP-38	PE-38	TGF- $\alpha$	Intratumoral (CED)	I	20 (17 GBM, other 3)	Median survival 28 weeks (95% CI, 4.1–45.1)	Hemiparesis, fatigue, headache, and dysphasia	[27, 28]
Tf-CRM107	DT-CRM107	Tf	Intratumoral (CED)	I/II	44 (GBM, AA)	Median survival 37 weeks, (95% CI, 26–49); 5/34 CR, 7/34 PR, response rate 35% (95% CI, 20–54; $P < 0.0001$ )	Seizure, cerebral edema	[29]

GBM: glioblastoma multiforme; AA: anaplastic astrocytoma; TGF: transforming growth factor; CED: convection-enhanced delivery; MTIC: maximum-tolerated infusate concentration; CI: confidence interval; Tf: transferrin; CR: complete response; PR: partial responders; RR: radiographic response.

Ricin-based immunotoxins are probably some of the most frequently studied immunotoxins to date. Clinical trials started as early as 1994, where ricin A chain conjugates as well as galactose binding site were used, blocked intact ricin conjugates, primarily focusing on hematological malignancies [116–118]. In metastatic brain tumors, an early clinical trial using a human TfR MAb conjugated to ricin A chain (454A12-rRA) was started administering this ricin A conjugated intrathecally to patients with carcinomatous meningitis with doses ranging from 1.2 to 1200  $\mu\text{g}$  [119, 120]. A cerebrospinal fluid (CSF) inflammatory response manifesting with headache, vomiting, and mental status change, occurred at doses  $\geq 120 \mu\text{g}$ . Four of the eight patients demonstrated a greater than 95% transient reduction in tumor cell counts in their CSF. One patient improved clinically, but none of the patients survived in the long term. In order to avoid the immunogenicity associated with bacterial or plant toxins, human cytotoxic proteins such as ribonuclease or granzyme B have been used to target endothelial cells in tumors or tumor cells [121]. Furthermore, the expression of cancer-related proteases provides the opportunity to convert toxins into precursor toxins by replacing the furin cleavage site with a protease expressed in cancer cells. For example, the toxin

is not active until it is cleaved by furin, and the furin site can be replaced by a site cleaved by urokinase using genetic mutation [122]. Several single-chain ribosome-inactivating proteins have also been used to make targeted toxins.

However, it is difficult to obtain adequate quantities of tumor-specific T cells, and the isolation and *ex vivo* clonal expansion of cytotoxic T lymphocytes (CTLs) from patients are a long and cumbersome process. As a result, a wide and general application of this approach has been limited. Many of the limitations associated with cellular immunotherapy can be circumvented by arming polyclonal CTL with tumor-specific chimeric T-cell receptors (TCR), the so-called “T-body” approach [15]. Chimeric TCR typically consist of a tumor-antigen-specific recognition scFv element derived from a mAb and components of TCR that mediate signal transduction in the CTL [16]. The T-body has the potential to recognize specific antigens in a major histocompatibility complex-(MHC-) independent manner; the applicability of this approach has been demonstrated both *in vitro* and *in vivo*.

In other studies have been used toxins that could regulate the immune system; however, a major problem with targeted toxins is the immunogenicity caused by the toxin. Pertussis

toxin (PTx), a well-known toxin isolated from *Bordetella pertussis*, exerts great activity modulating the immune system. Currently, several studies regarding the effects of PTx in cancer have been initiated. Recently, we developed a study where the pleiotropic effect of PTx in an experimental model of glioblastoma C6 was analyzed. We observed a significant decrease in tumor volume in the PTx group; this was associated with a decrease in the number of regulatory T cells (Treg) and an increase of apoptotic cells. The production of proinflammatory cytokines was increased in mRNA for IL-6; a small increase in the mRNA expression of perforin and granzyme was observed in tumors from rats treated with PTx as well. Even though this was the first study where PTx was used as adjuvant in the treatment of cancer, the toxin could have applications in the integral therapy against glial tumors [123].

## 6. Perspectives and Conclusion

The treatment of gliomas remains as a great challenge in the clinical response, free survival in patients, and inhibition of tumoral progression. Conventional methods for the treatment of brain tumors usually involve delivery of drugs via systemic circulation. High systemic drug levels are often required to achieve adequate drug concentrations at the site of the brain tumor, which usually requires increasing the dose, frequency, or duration of drug administration with the consequent systemic toxicity. The resistance to several treatments, toxicity, and early progression to malignancy has leading investigational studies for the development of specific antibodies to target tumoral cells and inhibit their growth. Another important failure in cancer therapy is due to sustained antitumor effects in the tumor microenvironment long enough to achieve clinically relevant therapeutic efficacy. At present, anti-glioma targeting therapy focuses on delivering specific drugs that inhibit the tumoral growth and elicit its deletion by immune system.

On the other hand, it is necessary to develop strategies that increase the ability of therapeutic antibodies to cross the brain blood barrier (BBB). The design of nanoparticles conjugates with antineoplastic antibodies offers high specificity, increasing the focal levels of drugs and eliciting the delivery of them into the tumor, which could decrease the adverse events produced by conventional systemic administration. Recently, a new approach in anticancer therapy is to conjugate drugs, such as cisplatin, into liposomes or nanoparticles that guarantee its free access through BBB eliciting high levels and permanence of drugs in tumoral sites. Moreover, decreasing the size of therapeutic antibodies to conjugate them to nanoparticles is a new approach to elicit their delivering into poorly accessible CNS tumors.

Another challenge in delivery techniques for the treatment of gliomas is the distribution of therapeutic antibodies into the solid tumors due to the differences encountered between the inner and outer levels of growth factors secreted by the tumor mass, causing the tumoral cells to have a particular response to the administered treatment depending on their location. Also, it has been observed that the hypoxia

levels are different in the central part than in the periphery of tumor; therefore, this hypoxia level mediates resistance to antiangiogenic therapy [25]. The bifunctional antibodies could be able to diffuse into the overall mass, diminishing the hypoxia levels by devascularization of tumor or by the use of antiangiogenic antibodies and by inducing an immune response to specific antineoplastic toxins.

## Disclosure

The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the paper apart from those disclosed.

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## References

- [1] L. M. Weiner, R. Surana, and S. Wang, "Monoclonal antibodies: versatile platforms for cancer immunotherapy," *Nature Reviews Immunology*, vol. 10, no. 5, pp. 317–327, 2010.
- [2] H. Ohgaki and P. Kleihues, "Genetic pathways to primary and secondary glioblastoma," *The American Journal of Pathology*, vol. 170, no. 5, pp. 1445–1453, 2007.
- [3] D. A. Reardon, M. J. Egorin, A. Desjardins et al., "Phase I pharmacokinetic study of the vascular endothelial growth factor receptor tyrosine kinase inhibitor vatalanib (PTK787) plus imatinib and hydroxyurea for malignant glioma," *Cancer*, vol. 115, no. 10, pp. 2188–2198, 2009.
- [4] E. R. Gerstner, A. F. Eichler, S. R. Plotkin et al., "Phase I trial with biomarker studies of vatalanib (PTK787) in patients with newly diagnosed glioblastoma treated with enzyme inducing anti-epileptic drugs and standard radiation and temozolomide," *Journal of Neuro-Oncology*, vol. 103, no. 2, pp. 325–332, 2011.
- [5] G. Neufeld, T. Cohen, S. Gengrinovitch, and Z. Poltorak, "Vascular endothelial growth factor (VEGF) and its receptors," *FASEB Journal*, vol. 13, no. 1, pp. 9–22, 1999.
- [6] R. K. Jain, D. G. Duda, J. W. Clark, and J. S. Loeffler, "Lessons from phase III clinical trials on anti-VEGF therapy for cancer," *Nature Clinical Practice Oncology*, vol. 3, no. 1, pp. 24–40, 2006.
- [7] H. M. Verheul and H. M. Pinedo, "The role of vascular endothelial growth factor (VEGF) in tumor angiogenesis and early clinical development of VEGF-receptor kinase inhibitors," *Clinical Breast Cancer*, vol. 1, supplement 1, pp. S80–84, 2000.
- [8] P. Traxler, P. R. Allegrini, R. Brandt et al., "AEE788: a dual family epidermal growth factor receptor/ErbB2 and vascular endothelial growth factor receptor tyrosine kinase inhibitor with antitumor and antiangiogenic activity," *Cancer Research*, vol. 64, no. 14, pp. 4931–4941, 2004.
- [9] E. Díaz-Rubio, "Vascular endothelial growth factor inhibitors in colon cancer," *Advances in Experimental Medicine and Biology*, vol. 587, pp. 251–275, 2006.
- [10] R. J. Kreitman, "Recombinant immunotoxins containing truncated bacterial toxins for the treatment of hematologic malignancies," *BioDrugs*, vol. 23, no. 1, pp. 1–13, 2009.

- [11] L. Greenfield, V. G. Johnson, and R. J. Youle, "Mutations in diphtheria toxin separate binding from entry and amplify immunotoxin selectivity," *Science*, vol. 238, no. 4826, pp. 536–539, 1987.
- [12] D. P. Williams, K. Parker, P. Bacha et al., "Diphtheria toxin receptor binding domain substitution with interleukin-2: genetic construction and properties of a diphtheria toxin-related interleukin-2 fusion protein," *Protein Engineering*, vol. 1, no. 6, pp. 493–498, 1987.
- [13] A. Shapira and I. Benhar, "Toxin-based therapeutic approaches," *Toxins*, vol. 2, no. 11, pp. 2519–2583, 2010.
- [14] C. B. Siegall, V. K. Chaudhary, D. J. FitzGerald, and I. Pastan, "Functional analysis of domains II, Ib, and III of *Pseudomonas* exotoxin," *Journal of Biological Chemistry*, vol. 264, no. 24, pp. 14256–14261, 1989.
- [15] I. Pastan, "Immunotoxins containing *Pseudomonas* exotoxin A: a short history," *Cancer Immunology, Immunotherapy*, vol. 52, no. 5, pp. 338–341, 2003.
- [16] R. Jørgensen, A. E. Purdy, R. J. Fieldhouse, M. S. Kimber, D. H. Bartlett, and A. R. Merrill, "Cholix toxin, a novel ADP-ribosylating factor from *Vibrio cholerae*," *Journal of Biological Chemistry*, vol. 283, no. 16, pp. 10671–10678, 2008.
- [17] R. Sarnovsky, T. Tendler, M. Makowski et al., "Initial characterization of an immunotoxin constructed from domains II and III of cholera exotoxin," *Cancer Immunology, Immunotherapy*, vol. 59, no. 5, pp. 737–746, 2010.
- [18] E. S. Vitetta, P. E. Thorpe, and J. W. Uhr, "Immunotoxins: magic bullets or misguided missiles?" *Immunology Today*, vol. 14, no. 6, pp. 252–259, 1993.
- [19] I. Pastan, R. Hassan, D. J. FitzGerald, and R. J. Kreitman, "Immunotoxin treatment of cancer," *Annual Review of Medicine*, vol. 58, pp. 221–237, 2007.
- [20] M. Mathew and R. S. Verma, "Humanized immunotoxins: a new generation of immunotoxins for targeted cancer therapy," *Cancer Science*, vol. 100, no. 8, pp. 1359–1365, 2009.
- [21] L. M. Budel, I. P. Touw, R. Delwel, S. C. Clark, and R. Lowenberg, "Interleukin-3 and granulocyte-monocyte colony-stimulating factor receptors on human acute myelocytic leukemia cells and relationship to the proliferative response," *Blood*, vol. 74, no. 2, pp. 565–571, 1989.
- [22] R. A. Messmann, E. S. Vitetta, D. Headlee et al., "A phase I study of combination therapy with immunotoxins IgG-HD37-deglycosylated ricin A chain (dgA) and IgG-RFB4-dgA (Combotox) in patients with refractory CD19(+), CD22(+) B cell lymphoma," *Clinical Cancer Research*, vol. 6, no. 4, pp. 1302–1313, 2000.
- [23] L. Herrera, B. Bostrom, L. Gore et al., "A phase I study of combotox in pediatric patients with refractory B-lineage acute lymphoblastic leukemia," *Journal of Pediatric Hematology/Oncology*, vol. 31, no. 12, pp. 936–941, 2009.
- [24] R. Schnell, E. Vitetta, J. Schindler et al., "Clinical trials with an anti-CD25 ricin A-chain experimental and immunotoxin (RFT5-SMPT-dgA) in Hodgkin's lymphoma," *Leukemia and Lymphoma*, vol. 30, no. 5-6, pp. 525–537, 1998.
- [25] R. Schnell, O. Staak, P. Borchmann et al., "A Phase I study with an anti-CD30 ricin A-chain immunotoxin (Ki-4.dgA) in patients with refractory CD30+ Hodgkin's and non-Hodgkin's lymphoma," *Clinical Cancer Research*, vol. 8, no. 6, pp. 1779–1786, 2002.
- [26] Y. Xu, Q. Xu, M. G. Rosenblum, and D. A. Scheinberg, "Antileukemic activity of recombinant humanized M195-gelolin immunotoxin in nude mice," *Leukemia*, vol. 10, no. 2, pp. 321–326, 1996.
- [27] A. Dean, M. Talpaz, H. Kantarjian et al., "Phase I clinical trial of the anti-CD33 immunotoxin HuM195/rgel in patients (pts) with advanced myeloid malignancies," *Journal of Clinical Oncology*, vol. 28, 15 supplement, 2010.
- [28] E. Olsen, M. Duvic, A. Frankel et al., "Pivotal phase III trial of two dose levels of denileukin diftitox for the treatment of cutaneous T-cell lymphoma," *Journal of Clinical Oncology*, vol. 19, no. 2, pp. 376–388, 2001.
- [29] K. Chang and I. Pastan, "Molecular cloning of mesothelin, a differentiation antigen present on mesothelium, mesotheliomas, and ovarian cancers," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 93, no. 1, pp. 136–140, 1996.
- [30] G. Giamas, Y. L. Man, H. Hirner et al., "Kinases as targets in the treatment of solid tumors," *Cellular Signalling*, vol. 22, no. 7, pp. 984–1002, 2010.
- [31] G. Carpenter and S. Cohen, "Epidermal growth factor," *Journal of Biological Chemistry*, vol. 265, no. 14, pp. 7709–7712, 1990.
- [32] G. J. Wikstrand, C. J. Reist, G. E. Archer, M. R. Zalutsky, and D. D. Bigner, "The class III variant of the epidermal growth factor receptor (EGFRvIII): characterization and utilization as an immunotherapeutic target," *Journal of NeuroVirology*, vol. 4, no. 2, pp. 148–158, 1998.
- [33] R. Nishikawa, X.-D. Ji, R. C. Harmon et al., "A mutant epidermal growth factor receptor common in human glioma confers enhanced tumorigenicity," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 91, no. 16, pp. 7727–7731, 1994.
- [34] H.-J. Su Huang, M. Nagane, C. K. Klingbeil et al., "The enhanced tumorigenic activity of a mutant epidermal growth factor receptor common in human cancers is mediated by threshold levels of constitutive tyrosine phosphorylation and unattenuated signaling," *Journal of Biological Chemistry*, vol. 272, no. 5, pp. 2927–2935, 1997.
- [35] Y. Zhang, F. Guessous, A. Kofman, D. Schiff, and R. Abounader, "XL-184, a MET, VEGFR-2 and RET kinase inhibitor for the treatment of thyroid cancer, glioblastoma multiforme and NSCLC," *IDrugs*, vol. 13, no. 2, pp. 112–121, 2010.
- [36] L. Frederick, X.-Y. Wang, G. Eley, and C. D. James, "Diversity and frequency of epidermal growth factor receptor mutations in human glioblastomas," *Cancer Research*, vol. 60, no. 5, pp. 1383–1387, 2000.
- [37] M. M. Feldkamp, P. Lala, N. Lau, L. Roncari, and A. Guha, "Expression of activated epidermal growth factor receptors, Ras-guanosine triphosphate, and mitogen-activated protein kinase in human glioblastoma multiforme specimens," *Neurosurgery*, vol. 45, no. 6, pp. 1442–1453, 1999.
- [38] D. R. M. Negri, E. Tosi, O. Valota et al., "In vitro and in vivo stability and anti-tumour efficacy of an anti-EGFR/anti-CD3 F(ab')<sub>2</sub> bispecific monoclonal antibody," *British Journal of Cancer*, vol. 72, no. 4, pp. 928–933, 1995.
- [39] K. Mishima, "Growth suppression of intracranial xenografted glioblastomas overexpressing mutant epidermal growth factor receptors by systemic administration of monoclonal antibody (mAb) 806, a novel monoclonal antibody directed to the receptor," *Cancer Research*, vol. 61, no. 14, pp. 5349–5354, 2001.
- [40] D. Patel, A. Lahiji, S. Patel et al., "Monoclonal antibody cetuximab binds to and down-regulates constitutively activated

- epidermal growth factor receptor vIII on the cell surface," *Anticancer Research*, vol. 27, no. 5, pp. 3355–3366, 2007.
- [41] J. H. Sampson, L. E. Crotty, S. Lee et al., "Unarmed, tumor-specific monoclonal antibody effectively treats brain tumors," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 97, no. 13, pp. 7503–7508, 2000.
- [42] B. Neyns, J. Sadones, E. Joosens et al., "Stratified phase II trial of cetuximab in patients with recurrent high-grade glioma," *Annals of Oncology*, vol. 20, no. 9, pp. 1596–1603, 2009.
- [43] L. Moserle, A. Amadori, and S. Indraco, "The angiogenic switch: implications in the regulation of tumor dormancy," *Current Molecular Medicine*, vol. 9, no. 8, pp. 935–941, 2009.
- [44] S. R. Wedge, J. Kendrew, L. F. Hennequin et al., "AZD2171: a highly potent, orally bioavailable, vascular endothelial growth factor receptor-2 tyrosine kinase inhibitor for the treatment of cancer," *Cancer Research*, vol. 65, no. 10, pp. 4389–4400, 2005.
- [45] K. Alitalo and P. Carmeliet, "Molecular mechanisms of lymphangiogenesis in health and disease," *Cancer Cell*, vol. 1, no. 3, pp. 219–227, 2002.
- [46] K. Okamoto, D. Neureiter, B. Alinger et al., "The dual EGF/VEGF receptor tyrosine kinase inhibitor AEE788 inhibits growth of human hepatocellular carcinoma xenografts in nude mice," *International Journal of Oncology*, vol. 33, no. 4, pp. 733–742, 2008.
- [47] D. A. Reardon, C. A. Conrad, T. Cloughesy et al., "Phase I study of AEE788, a novel multitarget inhibitor of ErbB- and VEGF-receptor-family tyrosine kinases, in recurrent glioblastoma patients," *Cancer Chemotherapy and Pharmacology*, vol. 69, no. 6, pp. 1507–1518, 2012.
- [48] T. T. Batchelor, P. Mulholland, B. Neyns et al., "Phase III randomized trial comparing the efficacy of cediranib as monotherapy, and in combination with lomustine, versus lomustine alone in patients with recurrent glioblastoma," *Journal of Clinical Oncology*, vol. 31, no. 26, pp. 3212–3218, 2013.
- [49] F. M. Iwamoto, K. R. Lamborn, H. I. Robins et al., "Phase II trial of pazopanib (GW786034), an oral multi-targeted angiogenesis inhibitor, for adults with recurrent glioblastoma (North American Brain Tumor Consortium Study 06-02)," *Neuro-Oncology*, vol. 12, no. 8, pp. 855–861, 2010.
- [50] D. Hanahan and J. Folkman, "Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis," *Cell*, vol. 86, no. 3, pp. 353–364, 1996.
- [51] K. Imai and A. Takaoka, "Comparing antibody and small-molecule therapies for cancer," *Nature Reviews Cancer*, vol. 6, no. 9, pp. 714–727, 2006.
- [52] H. Glen, S. Mason, H. Patel, K. Macleod, and V. G. Brunton, "E7080, a multi-targeted tyrosine kinase inhibitor suppresses tumor cell migration and invasion," *BMC Cancer*, vol. 11, article 309, 2011.
- [53] E. Bergsland, H. Hurwitz, L. Fehrenbacher et al., "A randomized phase II trial comparing rhuMab VEGF (recombinant humanized monoclonal antibody to vascular endothelial cell growth factor) plus 5-fluorouracil/leucovorin (FU/LV) to FU/LV alone in patients with metastatic colorectal cancer," *Proceedings of the American Society of Clinical Oncology*, vol. 19, abstract 939, 2000.
- [54] M. S. Gordon, K. Margolin, M. Talpaz et al., "Phase I safety and pharmacokinetic study of recombinant human anti-vascular endothelial growth factor in patients with advanced cancer," *Journal of Clinical Oncology*, vol. 19, no. 3, pp. 843–850, 2001.
- [55] G. Sledge, K. Miller, and W. Novotny, "A phase II trial of single agent Rhumab VEGF (recombinant humanized monoclonal antibody to vascular endothelial cell growth factor) in patients with relapsed metastatic breast cancer," *Proceedings of the American Society of Clinical Oncology*, vol. 19, abstract 5C, 2000.
- [56] R. F. DeVore, L. Fehrenbacher, and R. S. Herbst, "A randomized phase II trial comparing Rhumab VEGF (recombinant humanized monoclonal antibody to vascular endothelial cell growth factor) plus carboplatin/paclitaxel (CP) to CP alone in patients with stage IIIB/IV NSCLC," *Proceedings of the American Society of Clinical Oncology*, vol. 19, abstract 1896, 2000.
- [57] M. D. Siegelin, C. M. Raskett, C. A. Gilbert, A. H. Ross, and D. C. Altieri, "Sorafenib exerts anti-glioma activity in vitro and in vivo," *Neuroscience Letters*, vol. 478, no. 3, pp. 165–170, 2010.
- [58] M. R. Gilbert, "First-line bevacizumab in combination with standard of care not recommended for newly diagnosed glioblastoma," *Proceedings of the ASCO Annual Meeting*, 2013.
- [59] H.-P. Gerber, A. McMurtrey, J. Kowalski et al., "Vascular endothelial growth factor regulates endothelial cell survival through the phosphatidylinositol 3'-kinase/Akt signal transduction pathway: requirement for Flk-1/KDR activation," *Journal of Biological Chemistry*, vol. 273, no. 46, pp. 30336–30343, 1998.
- [60] P. Traxler, G. Bold, E. Buchdunger et al., "Tyrosine kinase inhibitors: from rational design to clinical trials," *Medicinal Research Reviews*, vol. 21, no. 6, pp. 499–512, 2001.
- [61] G. D. Shah, N. Loizos, H. Youssoufian, J. D. Schwartz, and E. K. Rowinsky, "Rationale for the development of IMC-3G3, a fully human immunoglobulin G subclass 1 monoclonal antibody targeting the platelet-derived growth factor receptor  $\alpha$ ," *Cancer*, vol. 116, supplement, no. 4, pp. 1018–1026, 2010.
- [62] W. K. Yung, H. Friedman, E. Jackson et al., "A phase I trial of PTK787/ZK22584 (PTK/ZK), a novel oral VEGFR TK inhibitor in recurrent glioblastoma," *Proceedings of the American Society of Clinical Oncology*, vol. 21, article 79a, 2002.
- [63] T. A. T. Fong, L. K. Shawver, L. Sun et al., "SU5416 is a potent and selective inhibitor of the vascular endothelial growth factor receptor (Flk-1/KDR) that inhibits tyrosine kinase catalysis, tumor vascularization, and growth of multiple tumor types," *Cancer Research*, vol. 59, no. 1, pp. 99–106, 1999.
- [64] L. Geng, E. Donnelly, G. McMahon et al., "Inhibition of vascular endothelial growth factor receptor signaling leads to reversal of tumor resistance to radiotherapy," *Cancer Research*, vol. 61, no. 6, pp. 2413–2419, 2001.
- [65] P. Vajkoczy, M. D. Menger, B. Vollmar et al., "Inhibition of tumor growth, angiogenesis, and microcirculation by the novel Flk-1 inhibitor SU5416 as assessed by intravital multi-fluorescence videomicroscopy," *Neoplasia*, vol. 1, no. 1, pp. 31–41, 1999.
- [66] K. Pietras, T. Sjöblom, K. Rubin, C.-H. Heldin, and A. Östman, "PDGF receptors as cancer drug targets," *Cancer Cell*, vol. 3, no. 5, pp. 439–443, 2003.
- [67] D. Matei, S. Kelich, L. Cao et al., "PDGF BB induces VEGF secretion in ovarian cancer," *Cancer Biology and Therapy*, vol. 6, no. 12, pp. 1951–1959, 2007.
- [68] K. Paz and Z. Zhu, "Development of angiogenesis inhibitors to vascular endothelial growth factor receptor 2. Current status and future perspective," *Frontiers in Bioscience*, vol. 10, pp. 1415–1439, 2005.
- [69] J. L. Spratlan, R. B. Cohen, M. Eadens et al., "Phase I pharmacologic and biologic study of ramucirumab (imc-1121b), a fully human immunoglobulin G1 monoclonal antibody targeting the vascular endothelial growth factor receptor-2," *Journal of Clinical Oncology*, vol. 28, no. 5, pp. 780–787, 2010.

- [70] D. A. Reardon, M. D. Groves, P. Y. Wen et al., "A phase I/II trial of pazopanib in combination with lapatinib in adult patients with relapsed malignant glioma," *Clinical Cancer Research*, vol. 19, no. 4, pp. 900–908, 2013.
- [71] J. Drappatz, A. D. Norden, E. T. Wong et al., "Phase I study of vandetanib with radiotherapy and temozolomide for newly diagnosed glioblastoma," *International Journal of Radiation Oncology Biology Physics*, vol. 78, no. 1, pp. 85–90, 2010.
- [72] C. Wibom, M. Sandström, R. Henriksson, H. Antti, M. Johansson, and A. T. Bergenheim, "Vandetanib alters the protein pattern in malignant glioma and normal brain in the BT4C rat glioma model," *International Journal of Oncology*, vol. 37, no. 4, pp. 879–890, 2010.
- [73] A. A. Brandes, R. Stupp, P. Hau et al., "EORTC study 26041-22041: phase I/II study on concomitant and adjuvant temozolomide (TMZ) and radiotherapy (RT) with PTK787/ZK222584 (PTK/ZK) in newly diagnosed glioblastoma," *European Journal of Cancer*, vol. 46, no. 2, pp. 348–354, 2010.
- [74] T. T. Batchelor, A. G. Sorensen, E. di Tomaso et al., "AZD2171, a pan-VEGF receptor tyrosine kinase inhibitor, normalizes tumor vasculature and alleviates edema in glioblastoma patients," *Cancer Cell*, vol. 11, no. 1, pp. 83–95, 2007.
- [75] T. T. Batchelor, D. G. Duda, E. Di Tomaso et al., "Phase II study of cediranib, an oral pan-vascular endothelial growth factor receptor tyrosine kinase inhibitor, in patients with recurrent glioblastoma," *Journal of Clinical Oncology*, vol. 28, no. 17, pp. 2817–2823, 2010.
- [76] D. A. Reardon, J. J. Vredenburgh, A. Desjardins et al., "Phase I trial of dasatinib plus erlotinib in adults with recurrent malignant glioma," *Journal of Neuro-Oncology*, vol. 108, no. 3, pp. 499–506, 2012.
- [77] M. G. McNamara and W. P. Mason, "Antiangiogenic therapies in glioblastoma multiforme," *Expert Review of Anticancer Therapy*, vol. 12, no. 5, pp. 643–654, 2012.
- [78] S. Bruheim, A. Kristian, T. Uenaka et al., "Antitumor activity of oral E7080, a novel inhibitor of multiple tyrosine kinases, in human sarcoma xenografts," *International Journal of Cancer*, vol. 129, no. 3, pp. 742–750, 2011.
- [79] R. H. Alvarez, H. M. Kantarjian, and J. E. Cortes, "Biology of platelet-derived growth factor and its involvement in disease," *Mayo Clinic Proceedings*, vol. 81, no. 9, pp. 1241–1257, 2006.
- [80] F. Yang, C. Brown, R. Buettner et al., "Sorafenib induces growth arrest and apoptosis of human glioblastoma cells through the dephosphorylation of signal transducers and activators of transcription 3," *Molecular Cancer Therapeutics*, vol. 9, no. 4, pp. 953–962, 2010.
- [81] J. D. Hainsworth, T. Ervin, E. Friedman et al., "Concurrent radiotherapy and temozolomide followed by temozolomide and sorafenib in the first-line treatment of patients with glioblastoma multiforme," *Cancer*, vol. 116, no. 15, pp. 3663–3669, 2010.
- [82] R. Abounader and J. Laterra, "Scatter factor/hepatocyte growth factor in brain tumor growth and angiogenesis," *Neuro-Oncology*, vol. 7, no. 4, pp. 436–451, 2005.
- [83] O. Arrieta, E. Garcia, P. Guevara et al., "Hepatocyte growth factor is associated with poor prognosis of malignant gliomas and is a predictor for recurrence of meningioma," *Cancer*, vol. 94, no. 12, pp. 3210–3218, 2002.
- [84] K. Lamszus, J. Laterra, M. Westphal, and E. M. Rosen, "Scatter factor/hepatocyte growth factor (SF/HGF) content and function in human gliomas," *International Journal of Developmental Neuroscience*, vol. 17, no. 5–6, pp. 517–530, 1999.
- [85] J. P. Eder, G. F. Vande Woude, S. A. Boerner, and P. M. Lorusso, "Novel therapeutic inhibitors of the c-Met signaling pathway in cancer," *Clinical Cancer Research*, vol. 15, no. 7, pp. 2207–2214, 2009.
- [86] C. F. Gao and G. F. Vande Woude, "HGF/SF-Met signaling in tumor progression," *Cell Research*, vol. 15, no. 1, pp. 49–51, 2005.
- [87] E. M. Rosen, J. Laterra, A. Joseph et al., "Scatter factor expression and regulation in human glial tumors," *International Journal of Cancer*, vol. 67, no. 2, pp. 248–255, 1996.
- [88] S. Koochekpour, M. Jeffers, S. Rulong et al., "Met and hepatocyte growth factor/scatter factor expression in human gliomas," *Cancer Research*, vol. 57, no. 23, pp. 5391–5398, 1997.
- [89] T. Moriyama, H. Kataoka, H. Kawano et al., "Comparative analysis of expression of hepatocyte growth factor and its receptor, c-met, in gliomas, meningiomas and schwannomas in humans," *Cancer Letters*, vol. 124, no. 2, pp. 149–155, 1998.
- [90] N. O. Schmidt, M. Westphal, C. Hagel et al., "Levels of vascular endothelial growth factor, hepatocyte growth factor/scatter factor and basic fibroblast growth factor in human gliomas and their relation to angiogenesis," *International Journal of Cancer*, vol. 84, no. 1, pp. 10–18, 1999.
- [91] J. Laterra, M. Nam, E. Rosen et al., "Scatter factor/hepatocyte growth factor gene transfer enhances glioma growth and angiogenesis in vivo," *Laboratory Investigation*, vol. 76, no. 4, pp. 565–577, 1997.
- [92] B. Cao, Y. Su, M. Oskarsson et al., "Neutralizing monoclonal antibodies to hepatocyte growth factor/scatter factor (HGF/SF) display antitumor activity in animal models," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 98, no. 13, pp. 7443–7448, 2001.
- [93] T. H. Nguyen, N. Loux, I. Dagher et al., "Improved gene transfer selectivity to hepatocarcinoma cells by retrovirus vector displaying single-chain variable fragment antibody against c-Met," *Cancer Gene Therapy*, vol. 10, no. 11, pp. 840–849, 2003.
- [94] T. Martens, N.-O. Schmidt, C. Eckerich et al., "A novel one-armed anti-c-Met antibody inhibits glioblastoma growth in vivo," *Clinical Cancer Research*, vol. 12, no. 20, pp. 6144–6152, 2006.
- [95] F. Bosch and L. Rosich, "The contributions of Paul Ehrlich to pharmacology: a tribute on the occasion of the centenary of his Nobel Prize," *Pharmacology*, vol. 82, no. 3, pp. 171–179, 2008.
- [96] R. J. Kreitman, "Immunotoxins for targeted cancer therapy," *AAPS Journal*, vol. 8, no. 3, article 63, pp. E532–E551, 2006.
- [97] S. Choudhary, M. Mathew, and R. S. Verma, "Therapeutic potential of anticancer immunotoxins," *Drug Discovery Today*, vol. 16, no. 11–12, pp. 495–503, 2011.
- [98] W. D. Janthur, N. Cantoni, and C. Mamot, "Drug conjugates such as antibody drug conjugates (ADCs), immunotoxins and immunoliposomes challenge daily clinical practice," *International Journal of Molecular Sciences*, vol. 13, no. 12, pp. 16020–16045, 2012.
- [99] R. E. Bird, K. D. Hardman, J. W. Jacobson et al., "Single-chain antigen-binding proteins," *Science*, vol. 241, no. 4877, pp. 423–426, 1988.
- [100] J. S. Huston, D. Levinson, M. Mudgett-Hunter et al., "Protein engineering of antibody binding sites: recovery of specific activity in an anti-digoxin single-chain Fv analogue produced in *Escherichia coli*," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 85, no. 16, pp. 5879–5883, 1988.

- [101] Y. Reiter, L. H. Pai, U. Brinkmann, Q.-C. Wang, and I. Pastan, "Antitumor activity and pharmacokinetics in mice of a recombinant immunotoxin containing a disulfide-stabilized Fv fragment," *Cancer Research*, vol. 54, no. 10, pp. 2714–2718, 1994.
- [102] A. M. Pappenheimer Jr., "Diphtheria toxin," *Annual Review of Biochemistry*, vol. 46, pp. 69–94, 1977.
- [103] M. Yamaizumi, E. Mekada, T. Uchida, and Y. Okada, "One molecule of diphtheria toxin fragment A introduced into a cell can kill the cell," *Cell*, vol. 15, no. 1, pp. 245–250, 1978.
- [104] P. Boquet, M. S. Silverman, and A. M. Pappenheimer Jr., "Interaction of diphtheria toxin with mammalian cell membranes," *Progress in Clinical and Biological Research*, vol. 17, pp. 501–509, 1977.
- [105] B. Varol, M. Bektaş, R. Nurten, and E. Bermek, "The cytotoxic effect of diphtheria toxin on the actin cytoskeleton," *Cellular & Molecular Biology Letters*, vol. 17, no. 1, pp. 49–61, 2012.
- [106] T. F. Liu, P. D. Hall, K. A. Cohen et al., "Interstitial diphtheria toxin-epidermal growth factor fusion protein therapy produces regressions of subcutaneous human glioblastoma multiforme tumors in athymic nude mice," *Clinical Cancer Research*, vol. 11, no. 1, pp. 329–334, 2005.
- [107] V. G. Johnson, D. Wilson, L. Greenfield, and R. J. Youle, "The role of the diphtheria toxin receptor in cytosol translocation," *Journal of Biological Chemistry*, vol. 263, no. 3, pp. 1295–1300, 1988.
- [108] M. Weaver and D. W. Laske, "Transferrin receptor ligand-targeted toxin conjugate (Tf-CRM107) therapy of malignant gliomas," *Journal of Neuro-Oncology*, vol. 65, no. 1, pp. 3–13, 2003.
- [109] P. J. Nicholls and R. J. Youle, "The structure of *Pseudomonas* exotoxin A as a guide to rational design," *Targeted Diagnosis and Therapy*, vol. 7, pp. 439–446, 1992.
- [110] T. Kondo, D. FitzGerald, V. K. Chaudhary, S. Adhya, and I. Pastan, "Activity of immunotoxins constructed with modified *Pseudomonas* exotoxin A lacking the cell recognition domain," *Journal of Biological Chemistry*, vol. 263, no. 19, pp. 9470–9475, 1988.
- [111] J. K. Batra, P. G. Kasprzyk, R. E. Bird, I. Pastan, and C. R. King, "Recombinant anti-erbB2 immunotoxins containing *Pseudomonas* exotoxin," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 89, no. 13, pp. 5867–5871, 1992.
- [112] U. Brinkmann, L. H. Pai, D. J. FitzGerald, M. Willingham, and I. Pastan, "B3(Fv)-PE38KDEL, a single-chain immunotoxin that causes complete regression of a human carcinoma in mice," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 88, no. 19, pp. 8616–8620, 1991.
- [113] P. C. Phillips, C. Levow, M. Catterall, O. M. Colvin, I. Pastan, and H. Brem, "Transforming growth factor- $\alpha$ -*Pseudomonas* exotoxin fusion protein (TGF- $\alpha$ -PE38) treatment of subcutaneous and intracranial human glioma and medulloblastoma xenografts in athymic mice," *Cancer Research*, vol. 54, no. 4, pp. 1008–1015, 1994.
- [114] R. W. Rand, R. J. Kreitman, N. Patronas, F. Varricchio, I. Pastan, and R. K. Puri, "Intratumoral administration of recombinant circularly permuted interleukin-4-*Pseudomonas* exotoxin in patients with high-grade glioma," *Clinical Cancer Research*, vol. 6, no. 6, pp. 2157–2165, 2000.
- [115] I. A. J. Lorimer, A. Keppler-Hafkemeyer, R. A. Beers, C. N. Pegram, D. D. Bigner, and I. Pastan, "Recombinant immunotoxins specific for a mutant epidermal growth factor receptor: targeting with a single chain antibody variable domain isolated by phage display," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 93, no. 25, pp. 14815–14820, 1996.
- [116] I. Pastan, R. Hassan, D. J. FitzGerald, and R. J. Kreitman, "Immunotoxin therapy of cancer," *Nature Reviews Cancer*, vol. 6, no. 7, pp. 559–565, 2006.
- [117] R. J. Kreitman and I. Pastan, "Immunotoxins in the treatment of hematologic malignancies," *Current Drug Targets*, vol. 7, no. 10, pp. 1301–1311, 2006.
- [118] D. A. Vallera and D. E. Myers, "Immunotoxins containing ricin," *Cancer Treatment and Research*, vol. 37, pp. 141–159, 1988.
- [119] L. A. Martell, A. Agrawal, D. A. Ross, and K. M. Muraszko, "Efficacy of transferrin receptor-targeted immunotoxins in brain tumor cell lines and pediatric brain tumors," *Cancer Research*, vol. 53, no. 6, pp. 1348–1353, 1993.
- [120] D. W. Laske, K. M. Muraszko, E. H. Oldfield et al., "Intraventricular immunotoxin therapy for leptomeningeal neoplasia," *Neurosurgery*, vol. 41, no. 5, pp. 1039–1051, 1997.
- [121] B. Dälken, U. Giesübel, S. K. Knauer, and W. S. Wels, "Targeted induction of apoptosis by chimeric granzyme B fusion proteins carrying antibody and growth factor domains for cell recognition," *Cell Death and Differentiation*, vol. 13, no. 4, pp. 576–585, 2006.
- [122] R. J. Abi-Habib, S. Liu, T. H. Bugge, S. H. Leppla, and A. E. Frankel, "A urokinase-activated recombinant diphtheria toxin targeting the granulocyte-macrophage colony-stimulating factor receptor is selectively cytotoxic to human acute myeloid leukemia blasts," *Blood*, vol. 104, no. 7, pp. 2143–2148, 2004.
- [123] M. Orozco-Morales, F.-J. Sánchez-García, P. Guevara-Salazar et al., "Adjuvant immunotherapy of C6 glioma in rats with pertussis toxin," *Journal of Cancer Research and Clinical Oncology*, vol. 138, no. 1, pp. 23–33, 2012.

## Review Article

# Pediatric Multiple Sclerosis: Current Concepts and Consensus Definitions

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Multiple sclerosis (MS), a chronic inflammatory autoimmune disease of the central nervous system (CNS) commonly diagnosed in adults, is being recognized increasingly in children. An estimated 1.7%–5.6% of all patients with MS have clinical symptoms before reaching the age of 18 years. In comparison with adults, the diagnosis of MS in children can be more difficult, being dismissed or misdiagnosed as other clinical disorders. Although adults and children share basic aspects of the disorder, children have distinctive clinical features, neuroimaging, laboratory, and courses of the disease. The 2010 McDonald criteria have simplified the requirements for establishing the diagnosis of MS and have been proposed to be applicable for the diagnosis of pediatric MS, mainly in children 12 years and older. This paper describes the distinctive features of common pediatric demyelinating disorders, including MS, and summarizes the most recent advances based on the available literature.

## 1. Introduction

Multiple sclerosis (MS) is a chronic inflammatory disease of autoimmune nature, characterized by demyelination and axonal loss. MS commonly affects young adults and is considered a rare occurrence in children younger than 18 years of age. However, several studies have indicated that at least 5% of the total population with MS is composed of pediatric patients [1, 2]. Within the pediatric age group, the incidence is highest in those between 13 and 16 years of age. A small, but important, subgroup is younger than 10 years of age [3].

In 2007, an international committee proposed provisional consensus definitions that included a range of clinical and laboratory findings to facilitate unification of criteria for accurate diagnosis and to encourage and promote clinical research in pediatric demyelinating disease [4]. The original definitions have been recently reviewed and updated [5]. These unified criteria have allowed for progress to be made in the advancement of understanding the etiology, clinical manifestations, course, and neuroimaging findings of pediatric MS and other demyelinating disorders of the central nervous system (CNS). However, recognizing distinctive features of different demyelinating disorders to achieve better diagnostic certainty and optimal treatment remain challenging.

## 2. Demographics

MS mainly affects individuals between the ages of 20 and 40 years, with a peak incidence at the age of 30 years. Population studies and case-control series show that between 1.7 and 5.6% of the MS population is younger than 18 years of age [1, 2, 6, 7] and that onset before 10 years of age occurs in less than 1% of all multiple sclerosis cases [2, 7]. The global incidence of pediatric MS is unknown, and the few epidemiological studies exhibit variable results. In a California pediatric cohort, the reported incidence was approximately 0.51 per 100,000 people years [8]. A Canadian surveillance study of initial demyelinating events occurring in subjects younger than 18 years of age, including the first event of MS, neuromyelitis optica (NMO), optic neuritis (ON), acute disseminated encephalomyelitis (ADEM), and transverse myelitis (TM), yielded an incidence of 0.9 per 100,000 people [9]. Another nationwide prospective study in The Netherlands reported an annual incidence of ADS of 0.66/100,000 [10]. Epidemiological studies have determined that the place of residence during childhood is a determinant factor for the development of MS. Adolescent and younger immigrants less than 15 years of age acquire the MS risk that exists in the area to which they move, especially when

they move from areas where MS is rare to regions of high prevalence [11].

With regard to gender in pediatric MS, the ratio varies when age is taken into account. In subjects older than 10 years of age and adolescents, females predominate from 2.1:1 to 3:1, respectively. However, for those younger than 10 years of age, the female-to-male ratio ranges from 0.8:1 in children younger than 6 years of age to 1.6:1 in patients between 6 and 10 years of age [12].

Unlike the adult population, in whom MS usually affects non-Hispanic whites, pediatric MS shows greater racial and ethnic variability in North America. Chitnis et al. [13] reported not only a greater percentage of African American pediatric patients at a clinic in Boston compared with adults (7.4% versus 4.3%, resp.), but also a more severe clinical presentation for this ethnic group. At a center in Canada, most of the pediatric patients with MS had diverse ethnic backgrounds, including Caribbean, Asian, or Central and Eastern European [11]. The reasons for this ethnic and racial diversity have not been fully elucidated; however, various influences of genetic and environmental, as well as migration, with changing regional demographics factors, may play a role in North America [8, 14, 15]. Whether environmental risk factors for MS are becoming more prevalent during childhood among certain ethnicities or a shift is reflected in the ethnic distribution of general populations from which these cohorts were obtained remains unknown. The population-based cohort study of Southern California children showed a higher incidence of MS in black compared with white and Hispanic children, suggesting that the prevalence of environmental or genetic risk factors may be more common in black children [8].

Other potential environmental factors that contribute to the occurrence of MS include inadequate exposure to sunlight, vitamin D deficiency, viral infections, and exposure to cigarette smoke [16–31].

Usually, MS occurs more commonly in temperate regions, where exposure to ultraviolet light is limited [16]. Ultraviolet radiation is known to induce the synthesis of intraepithelial vitamin D. Currently, vitamin D is considered to be a powerful hormone involved in multiple biological processes, including self-immune recognition. 1,25-Dihydroxyvitamin D<sub>3</sub>, the active form of vitamin D, is a potent immunomodulator that plays key roles in innate and acquired immunities [17]. It downregulates dendritic cells and prevents the proliferation and enhances apoptosis of activated B cells [18, 19]. Lower levels of vitamin D have additionally been associated with increased risk of relapse among patients with relapsing-remitting MS (RRMS) or clinically isolated syndrome (CIS) [19]. In one recent study of pediatric MS, researchers found a 34% decrease in attacks for every 10 ng/mL increase in the level of circulating vitamin D [20]. Similarly, another study showed that each 10 ng/mL higher level of 25-hydroxy vitamin D was associated with a 15% lower risk of acquiring a new T2 lesion and a 32% lower risk of acquiring a gadolinium-enhancing lesion [21].

The pediatric population presents a unique opportunity to study the role of viruses in the development of MS, given the lower total number of pathogen exposures in a young

host relative to adults. In addition, the serial novel exposure of children to common viral antigens and close temporal relationship between infection and the onset of pediatric MS provide opportunities to discover the relationship between disease and pathogen [14]. The shorter time lag between putative exposures and disease onset in pediatric MS patients may provide insight into specific environmental factors and/or a particular genetic susceptibility in the pediatric MS population. Viral infections, particularly remote infections with the Epstein-Barr virus (EBV), have been consistently associated with MS in adults and recently documented in more than 85% of children with MS [22, 23]. Banwell et al. [24] compared 137 children with definite MS and controls of the same age and found no differences between the two groups with respect to seropositivity to cytomegalovirus (CMV), herpes simplex type 1 virus, varicella zoster (VZ), and parvovirus B19. In contrast, EBV seropositivity was associated with an increased risk of developing MS in childhood. Another study with 147 children suffering from MS also showed EBV seropositivity more prevalent in patients than in controls (99% versus 72%,  $P = 0.001$ ) [25]. Numerous observations have supported the possibility of multifaceted gene-environment interactions, although only a few have been reported for MS, and those are unconfirmed. The strongest genetic risk factor for MS, HLA-DRB1, is a coreceptor for EBV entry into B cells. In a recent retrospective study, EBNA-1 was associated with increased odds for developing MS in analyses adjusted for age, sex, race, ethnicity, and *HLA-DRB1\*1501/1503*; a remote infection with CMV was associated with a lower risk of developing MS [26]. These findings suggest that a complex interplay may exist between various viral infections acquired during childhood and the risk of developing MS. The combined results of these studies do not yet establish if EBV and/or other infections predispose one to contract MS or if a shared immunogenetic susceptibility toward a symptomatic infection and MS may exist. Moreover, common environmental factors also may trigger both infectious mononucleosis and MS [27]. Further studies are needed to better identify risk factors for MS susceptibility and their interactions, which might lead to development of individualized preventive strategies and new treatments.

The role of some immunizations, especially hepatitis B vaccine, and the subsequent development of MS also have been investigated. Mikaeloff et al. [28] in a French study found no evidence of increased risk of developing a first episode of MS up to 3 years after receiving vaccination. In a second study by the same authors, no evidence was found of any increased rate of relapse after a first demyelinating event when patients were subsequently vaccinated against hepatitis B or tetanus [29]. In a carefully performed case-control analysis, these investigators [30] showed a trend for the Engerix B vaccine to increase the risk of MS in the long term. This did not reach statistical significance, and these results require confirmation.

The same research group assessed the likelihood of developing MS after passive exposure to cigarette smoke in French children. They compared 129 children with MS with 1,038 controls by age, sex, and place of residence. The authors found that the risk of having a first episode of MS in

individuals exposed to smoking habits of parents was more than twice that observed in individuals whose parents were nonsmokers, and this risk was even greater in those with prolonged exposure of 10 or more years [31].

### 3. Etiology

As with certain autoimmune diseases, the trigger mechanism of MS in childhood is unknown. The etiology of MS is thought to reflect a complex interplay between host genetic factors and environmental exposures. Still to be determined is how the various factors involved lead to the resulting demyelination and axonal loss that correlate with progression of the disease and neurologic disability. At this point, the literature offers some leading theories that attempt to explain the pathophysiological changes that cause MS. For instance, the largest genome-wide genetic association screens have revealed multiple disease-associated genes that are involved in the immune system function [32, 33]. The major histocompatibility complex exerts the greatest influence on the risk of developing MS followed by other immune genes. Traditionally, T cells were considered the main factor responsible for the attack against CNS elements, particularly myelin. The most recent evidence has revealed a more complex picture in which B cells, antibodies, and the innate immunity also participate in the tissue damage that involves not only myelin but also axons, cortical neurons, and nodes of Ranvier [34]. Despite the sufficient body of evidence on the pathology and neurobiology of MS, the precise characterization of the mechanisms involved in the pathogenesis of MS raises more questions than answers. Autoimmune targets of this widespread injury remain unknown, and one of the current unsolved questions is whether the primary autoimmune attack is the initial trigger (“outside-in model”) or if the MS process begins with a cytodegeneration focused on the oligodendrocyte-myelin complex that results in a reactive inflammatory CNS disorder (“inside-out model”) [35]. The current body of scientific information is consistent with either model, but the need is to understand how these key components work, taking into account the implications for therapeutic design.

Compared to the adult population, few studies in pediatric MS have examined markers of axonal damage in the cerebrospinal fluid (CSF). However, Rostasy et al. [36] presented a group of pediatric patients with MS clinical symptoms displaying elevated levels of Tau protein in the CSF, indicating increased damage to the CNS. The discovery of autoantigens that are expressed by both glial and neuronal cells indicates that an immune attack originally directed against the glial component also can target the neuronal component and vice versa in early events in the human disease [36]. Recent reports have identified autoantibodies to the axoglial membrane proteins neurofascin and contactin in patients with established RRMS [37–39]. More recently, in a study of CSF samples collected from children during initial presentation of acute demyelinating syndromes, levels of nodal/paranodal assembling proteins were significantly higher in the children who ultimately developed MS

compared to the monophasic group [40]. These findings complement the view that, as in adults, axoglial apparatus molecules have utility as biomarkers of MS injury and are implicated in early disease mechanisms [40]. A dysfunction of the axoglial interactions possibly leads to loss of trophic support for oligodendrocytes, which in turn may express stress proteins that incite a targeted immune response [40, 41]. Intensive efforts are needed in the field of biomarkers to improve the diagnosis, determine prognostic factors, and identify markers to monitor the clinical course and response to disease-modifying therapies [42]. The ability to perform in-depth analyses of genomes, transcriptomes, proteomes, and metabolomes remains a promising avenue for discoveries of biomarkers in MS.

### 4. Diagnosis

*4.1. First Demyelinating Event (Clinically Isolated Syndrome (CIS)).* The diagnosis of MS in children is a process that begins with a first event of acute demyelination. Hence, it is highly advisable to determine whether the patient will develop subsequent events compatible with MS or if the event is a self-limited disorder. The first attack of demyelination, termed *clinically isolated syndrome* (CIS) or *acquired demyelinating syndrome*, is characterized by a clinical monofocal or polyfocal episode of presumed inflammatory demyelinating cause with acute or subacute onset in the absence of encephalopathy that cannot be explained by fever or systemic illness and that does not meet the 2010 MS McDonald criteria on a baseline MRI [5, 43] (as shown in List 1). CIS can be characterized as clinically monofocal, affecting a localized part of the CNS (ON, brainstem syndrome; TM, hemispheric syndrome), or clinically polyfocal (localizing to multiple sites in the CNS). In a published series of 117 children with acute demyelination and initial monofocal symptoms, 43% were diagnosed with MS, compared to 21% of children with polyfocal features after a follow-up period of 54 months [44]. The likelihood of developing MS following a first event is extremely low in children with an otherwise normal brain MRI [5, 45, 46].

*List 1: Clinical Criteria for Pediatric MS and CNS Demyelinating Disorders [5]*

#### *Pediatric Clinically Isolated Syndrome (CIS)*

- (i) A monofocal or polyfocal clinical neurological event with presumed inflammatory demyelinating cause.
- (ii) Absence of encephalopathy that cannot be explained by fever.
- (iii) Absence of previous clinical history of CNS demyelinating disease.
- (iv) Other etiologies have been excluded.
- (v) The most recent 2010 revised MS McDonald criteria on a baseline MRI are not met.

#### *Monophasic ADEM*

- (i) A first polyfocal clinical neurological event with presumed inflammatory cause.

- (ii) Encephalopathy that cannot be explained by fever is present.
- (iii) No new symptoms, signs, or MRI findings after three months of the incident ADEM.

#### *Multiphasic ADEM*

- (i) A new event of ADEM three months or more after the initial event.
- (ii) Can be associated with new or reemergence of prior clinical and MRI findings.
- (iii) Timing in relation to steroids is no longer relevant.

#### *Pediatric Multiple Sclerosis*

- (i) Two or more clinical events separated by more than 30 days and involving more than one area of the CNS.
- (ii) A single clinical event plus a baseline MRI evidence for DIS and DIT that meets the recent 2010 revised McDonald criteria.
- (iii) ADEM followed more than three months later by a nonencephalopathic clinical event with new lesions on brain MRI consistent with MS.

#### *NMO*

All required

- (i) optic neuritis,
- (ii) acute Myelitis.

At least two of these three criteria are considered:

- (i) MRI evidence of a contiguous spinal cord lesion (3 or more segments in length),
- (ii) brain MRI nondiagnostic for MS,
- (iii) antiaquaporin-4 IgG seropositive status.

**4.2. Optic Neuritis.** Although ON in children may appear as a clinically isolated syndrome, other cases of ON are associated with ADEM, MS, NMO, and various other disorders, including inflammatory and infectious conditions. Alternatively, certain genetic conditions, vascular malformations, and compressive orbital tumors can mimic the features of an inflammatory optic neuropathy, necessitating careful investigation. Accordingly, the initial workup should be extensive, including neuroimaging and serologic studies to facilitate the differentiation. Imaging of the brain and orbits with MRI using specific sequences including T2-weighted orbital fat suppression can support the diagnosis of ON with hyperintensity and enlargement of the affected optic nerve. Optic nerve enhancement on T1-weighted sequences following administration of gadolinium is also consistent with an acute inflammatory event.

ON can be unilateral or bilateral. In one study, unilateral ON was observed in 58% of children, compared with a bilateral involvement in 42% of cases [45]. Although initial visual loss was severe in nearly 70% of this group of pediatric patients, 83% of them attained an excellent visual recovery

(better than 20/40). As previously noted, ON may occur in isolation as a monofocal clinically isolated syndrome, or it may be associated with other polyfocal acquired demyelinating disorders.

The risk of developing MS after having an isolated episode of ON in childhood has been reported to range between 10% and 56% [45, 47]. Many factors, including the absence of unified definitions, access to neuroimaging, small number of patients, and duration of followup, may explain these widely differing figures. Retrospective case series have examined the prognostic use of magnetic resonance imaging (MRI) in the development of MS following ON. For instance, in the Wilejto et al. study [45] of 36 children with ON, the presence of one or more white matter lesions extrinsic to the optic nerves was associated with a 68% risk for developing MS during the next 2.4 years. More recently, Alper and Wang [48] reported that 23% of pediatric patients with ON eventually developed MS within 6 years in their study and found a strong correlation between a normal MRI and a monophasic clinical presentation. For example, MS was diagnosed in 42% of children with an abnormal MRI, whereas 93% of children with normal MRIs remained relapse-free. Consequently, the presence of ON and associated MRI abnormalities increases the likelihood of developing MS.

**4.3. Acute Transverse Myelitis.** TM may manifest as a monofocal clinically isolated syndrome or be associated with ON, ADEM, or as a component of polyfocal clinically isolated syndrome. TM can be either segmental with involvement of individual vertebral segments of the spinal cord or longitudinally extensive, which is defined as acute transverse myelitis involving 3 or more continuous spinal cord segments in length. The outcome in children with TM is variable. In several series, a complete recovery was reported in 33% to 50% of patients and poor prognosis in approximately 10% to 20% of cases [49, 50].

The risk of MS developing in patients with isolated TM is low. Only one of 47 children with TM followed for a period of 8 years had MS [51]. In the Canadian prospective study, 21% of the children with acquired demyelinating syndrome presented with acute TM, which represented the first clinical event in approximately 10% of children with MS [9]. Although acute TM is a rare presenting symptom in pediatric MS, those children displaying patchy hyperintense T2 signals between 1 and 3 spinal segments or oligoclonal bands in the CSF have the greatest risk for developing MS within this group [49–51].

**4.4. Acute Disseminated Encephalomyelitis.** ADEM, defined as polyfocal neurological deficits of presumed inflammatory and demyelinating cause in association with encephalopathy, is usually a monophasic event [4]. This disorder affects mainly children younger than 10 years of age and usually occurs after they have had viral infections or rarely in association with recent vaccination. A comprehensive workup, including studies of infectious and neurometabolic causes, neuroimaging of the brain and spinal cord, analysis of the CSF, and neuroimmune tests, may help to differentiate ADEM from

other disorders [47, 52]. After an ADEM event occurs, the clinical manifestations and neuroimaging findings can fluctuate during the next 3 months and are considered to be part of the same event, rather than separate events. The occurrence of a second event characterized by clinical encephalopathy plus polyfocal neurological deficits at least 3 months after the first episode irrespective of steroid treatment is characterized as multiphasic disseminated encephalomyelitis (MDEM) [5]. Relapsing disease that follows ADEM beyond a second encephalopathic event currently suggests a chronic disorder that often predates the diagnosis of MS or NMO [53, 54]. Some studies have suggested that 18% to 29% of patients with ADEM as their first demyelinating attack progress to MS [47, 55]. However, in a recent prospective study following the definitions proposed by the International Pediatric Multiple Sclerosis Study Group (IPMSSG) on children with ADEM, only 6% developed MS in a 9-year followup [53].

Typical MRI characteristics of ADEM are large, usually at least 2 cm, hyperintense asymmetric lesions, disseminated and confluent, involving white matter, cortex, and the deep grey nuclei with gadolinium enhancement. Recently Callen et al. [56] proposed several MRI findings to better differentiate ADEM from MS. Most patients with ADEM show (a) a diffuse bilateral pattern, (b) absence of black holes, and (c) fewer than two periventricular lesions (sensitivity 81%, specificity 95%). As a consequence, the diagnosis of ADEM is based only on the combination of clinical and neuroimaging findings and exclusion of disorders that resemble this entity.

In children younger than 12 years with features of ADEM to include encephalopathy and polyfocal neurological deficits, application of the revised 2010 MS McDonald criteria for dissemination in space and time on initial MRI is considered inappropriate, and continued follow-up of clinical and MRI findings is needed to confirm a diagnosis of MS [5].

**4.5. Neuromyelitis Optica (NMO).** Neuromyelitis optica (NMO) is an uncommon inflammatory demyelinating disorder characterized by severe acute transverse myelitis (TM) with simultaneous or sequential unilateral or bilateral optic neuritis (ON). Usually reported in adults and rarely in children, NMO has been considered an exceptional manifestation of multiple sclerosis (MS). However, the discovery of a highly specific aquaporin-4 (AQP4) autoantibody (AQP4-IgG) has demonstrated that NMO is a distinct pathophysiological disorder [57].

Over the last five years a better understanding of pediatric NMO has emerged. A median age of onset of 10–14 years and strong female predominance have been observed [54, 57–59]. Pediatric NMO spectrum can either be monophasic or manifest clinical relapses of ON or TM. Relapsing attacks of ON and TM separated in time are more common, and up to 80% of this group of patients is AQP4-IgG seropositive. Relapsing NMO tends to progress more slowly in children than in adults [60], and clinical relapses of NMO can resemble features of ADEM to include the presence of encephalopathy and large hemispheric lesions on MRI [54, 61].

Diagnostic workup for NMO includes brain and spinal cord MRI and serum AQP4-IgG testing which is 99% specific

and 60%–70% sensitive even in pediatric patients [62]. Forty-two percent of children with features of NMO may display serologic (76%) or clinical evidence of systemic lupus erythematosus, Sjogren syndrome, or other autoimmune diseases [58].

Standard CSF analysis during an NMO attack may show pleocytosis with significant number of neutrophils and eosinophils and/or elevation of proteins; oligoclonal bands are generally absent [57, 60, 61].

Current diagnostic criteria are summarized in List 1. Features that suggest NMO or an NMO-spectrum disorder include (1) presence of longitudinally T2-hyperintense spinal cord lesions extending for greater than 3 vertebral segments, (2) optic neuritis, which may have a greater risk of residual deficit compared to ON associated with MS, and (3) brainstem symptoms to include intractable nausea/vomiting, vertigo, hearing loss, facial weakness, trigeminal neuralgia, diplopia, ptosis, and nystagmus [5].

**4.6. Pediatric Multiple Sclerosis.** According to international consensus clinical criteria, pediatric MS is defined by multiple episodes of demyelination of the CNS separated by time and space as specified in adults, eliminating any lower age limit [4, 5]. Therefore, pediatric MS can be diagnosed in patients younger than 18 years with two episodes of CNS demyelination separated by more than 30 days and involving more than one area of the CNS. The consensus is that clinical and radiological criteria of dissemination in time and space must be met [5, 43]. In children aged 12 years and older presenting with an acute event, some typical findings on a baseline MRI may facilitate establishing an early diagnosis when the observed changes are consistent with dissemination in space and time [5, 43].

A high sensitivity (84%) and specificity (93%) of T1 hypointense lesions and T2 periventricular lesions have been recently confirmed and validated as strong early predictors of MS diagnosis in children with acquired demyelinating syndrome (ADS) [63]. As noted earlier, most children with a single demyelinating attack of the CNS will not have recurrences, and only the assessments of clinical investigations, such as neuroimaging, analyses of the CSF, and other laboratory tests, can provide more accurate information regarding which children are at higher risk for developing MS among those who have a single monophasic event. The objective demonstration of dissemination of lesions in both space and time, based on either clinical findings alone or a combination of clinical and MRI findings, remains the core requirement for establishing the diagnosis of MS (List 1).

Most patients with pediatric MS present with a relapsing-remitting course and have much higher relapse rates compared to adults. Gorman et al. [64] have reported that the annualized relapse rate in the pediatric group was significantly higher than that in the adult-onset group (1.13 versus 0.40;  $P < 0.001$ ). This higher rate of early relapses in pediatric MS may be related to different immune activation or levels of cells and cytokines in the CNS. However, the result may have been influenced by referral, since large tertiary referral centers may see patients with a more aggressive

disease course. Further prospective studies of early relapse rate in children from first attack are required. Adolescents generally have a second clinical attack within 12 months after the first event, whereas the younger children have a greater time interval between the first and second attacks [7]. Most pediatric patients have complete recovery after their first attacks. A higher risk of experiencing permanent disability seems to be linked to the increased relapse rate within the first 2 years of the disease in pediatric patients [1, 7]. In general, disease progression is slower in pediatric MS, recovery after a clinical exacerbation is shorter in children compared to adults, and a lower proportion of children are classified with progressive forms of the disease [1, 7].

*4.7. Cognitive Impairment.* Available data suggest that approximately one-third of children and adolescents with MS experience cognitive impairment, defined as having at least one-third of completed test scores falling 1 standard deviation or more below published normative data. Areas of cognitive deficit can vary but often include attention and speeded processing, visuomotor functions, memory, and language [47, 65, 66]. Receptive language and verbal fluency are often more affected in pediatric compared with adult MS patients in whom the aspects of language are usually preserved. Interestingly, cognitive impairment was identified in 65 (35%) of 187 children with multiple sclerosis and 8 of 44 (18%) with clinically isolated syndrome in the largest sample studied to date [65]. The most frequent areas involved were fine motor coordination (54%), visuomotor integration (50%) and speeded information processing (35%). This relatively increased proportion of impairment in pediatric MS patients compared to CIS is consistent with the observation that cognitive impairments in children with multiple sclerosis progress over time [67]. Furthermore, the striking difference of cognitive impairment in the early disease course between children and adults with MS may be due to the effects of the inflammatory demyelinating process on the ongoing myelination in the developing brain and neuronal networks [47, 65].

Cognitive dysfunction is a major feature of pediatric multiple sclerosis that can occur at the earliest stages of the disease, interfering with the child's present and future academic performance. In addition, fatigue, depression, and reduced quality of life are important issues in pediatric demyelinating disorders and may occur at a rate up to three times that of controls [66, 67]. Depression or anxiety is present in 50% of children and adolescents with multiple sclerosis, thus interfering with their quality of daily life [65, 66]. Periodic neuropsychological and psychiatric assessment along with the development of interventions for cognitive decline, fatigue, and depression is warranted as part of routine care [68].

## 5. Differential Diagnosis

The diagnosis of pediatric MS is a clinical one, requiring the presence of recurrent episodes of CNS demyelination with supportive ancillary paraclinical data in the absence of

another plausible diagnosis. Neuroimaging and CSF analysis features help to establish the diagnosis of pediatric MS. Accordingly, before giving a patient a diagnosis of MS, clinicians should rule out other disorders that may display similar symptoms to include vascular, inflammatory, infectious, metabolic, and neurodegenerative disorders. In a prospective cohort of 332 children meeting clinical criteria for ADS, 20 (6%) were ultimately diagnosed with non-demyelinating disorders [69]. Clinical and paraclinical findings that suggest an alternative diagnosis to initial presentation of MS include fever, encephalopathy, progressive clinical course, involvement of the peripheral nervous system or other organ systems, increased leukocyte count or ESR, markedly elevated pleocytosis or proteinorraquia, and the absence of CSF oligoclonal bands [70]. The combination of peripheral neuropathy and CNS demyelination argue against MS and favor other entities such as leukodystrophies or mitochondrial diseases.

This group of disorders usually exhibits progressive neurologic deterioration in absence of a clear relapsing-remitting disease.

CNS vasculitis is a challenging differential diagnosis of ADS with occasional overlapping features to include optic neuritis, transverse myelitis, and polyfocal supratentorial and infratentorial neurologic deficits [69, 71]. Persistent headache, rarely observed in MS or children with CIS, was present in 4 of the 5 patients with childhood primary angiitis of the CNS in the O'Mahony et al.'s study [69]. Seizures were observed in 4 of the 5 children with childhood primary angiitis compared to only 3 of more than 301 children with CNS demyelination. Focal seizures in the absence of persistent neurological deficits may be associated with CNS malignancy.

The evolution of disease by neuroimaging can help to confirm or exclude an MS diagnosis. White matter abnormalities on MRI in pediatric patients have a wide range of differential diagnoses (List 2). These entities should more often be considered in the younger child or when the presentation is atypical [70].

### *List 2: Diagnostic Categories to Exclude in Pediatric Multiple Sclerosis*

#### *Vascular/Inflammatory Disease*

- (i) CNS vasculitis/childhood primary CNS angiitis,
- (ii) Stroke,
- (iii) CADASIL,
- (iv) Autoimmune disease: systemic lupus erythematosus, antiphospholipid antibody syndrome, neurosarcoïdosis, Sjogren's syndrome,
- (v) Migraine.

#### *Metabolic/Nutritional*

- (i) Mitochondrial encephalopathy,
- (ii) Leukodystrophies,
- (iii) B12 or folate deficiency.

### *CNS Infection*

- (i) Neuroborreliosis,
- (ii) Herpes simplex encephalitis,
- (iii) Influenza ANE,
- (iv) Viral encephalitis.

### *Malignancy*

- (i) Lymphoma,
- (ii) Astrocytoma.

Regarding neuroimaging, persistent lesion enhancement and edema or increasing size of lesions over time are considered “red flags” for an underlying malignant condition. Leptomeningeal enhancement uncommon in MS may point to either childhood primary angiitis of the CNS or an inflammatory disorder with meningeal infiltration as neurosarcoidosis [69]). Alternatively, clinical and imaging involvement of basal ganglia is not common in MS and may suggest other diagnosis such as mitochondrial disease, infectious encephalitis, or histiocytosis [69, 70]. A correct and timely diagnosis is vital to lead the children and their families to the appropriate treatment and reduce the potential long-term disability [70].

## **6. Diagnostic Tools**

In patients with demyelinating events, evaluation by neuroimaging, analysis of the CSF, visual testing including the visual evoked potentials (VEP), and ocular coherence tomography (OCT) are important diagnostic tools.

**6.1. Neuroimaging (Brain MRI).** Currently, MRI is the most important diagnostic tool for evaluating MS in both children and adults, as it has invaluable utility in the recognition of other disorders that may resemble ADEM or MS. MRI findings in MS consist of plaques of demyelination particularly visible on T2-weighted sequences and typically located in the deep white matter, corpus callosum, periventricular zone, and brainstem. T1 sequences may demonstrate “black holes” or hypointense lesions that represent complete tissue loss resulting from a previous inflammatory event (Figures 1(a)–1(f)). Enhancement of active areas of inflammation and blood-brain barrier compromise can be displayed with T1 gadolinium contrast sequences. Tumefactive T2-bright lesions can be seen in up to 0.3 cases per 100,000 per year. Characteristic features that can help to distinguish demyelination from a malignant process include preferential enhancement of the lesional rim facing the lateral ventricles [72, 73].

Retrospective data suggest that children at MS onset have a higher number of total hyperintense T2 lesions in the posterior fossa and overall more gadolinium-enhancing lesions than adults do. In addition, compared to adults, pediatric MS patients tend to have greater resolution of the initial T2 lesion burden on follow-up MRI, suggesting better recovery of demyelination in children [74].

Current diagnostic criteria for MS admit MRI evidence of new lesions over time to substitute for clinical relapses. The most recent revision of the McDonald criteria specifically outlines the applicability for the use of the revised criteria in children and permits the diagnosis of pediatric MS at a first clinical event [43]. According to these criteria, dissemination in space (DIS) can be fulfilled with one or more lesions in at least two of four CNS areas (periventricular, juxtacortical, infratentorial, or spinal cord). Additionally, DIT can also be fulfilled in patients with typical acute demyelinating syndrome with a single MRI study that demonstrates simultaneous presence of asymptomatic gadolinium-enhancing and nonenhancing lesions [5, 43].

In the prospective cohort study by Sadaka et al. [75], the 2010 revised McDonald criteria demonstrated high sensitivity (100%), specificity (86%), positive predictive value (76%), and negative predictive value (100%) for children older than 12 years with non-ADEM presentations. In younger children, these criteria are of less predictive value and not appropriate for application in the context of ADEM-like presentations.

The emerging emphasis on the MRI features in the diagnosis of MS in younger children can be challenging, given the higher incidence of ADEM in this age group and often equivalent imaging features between ADEM and MS in this population with large confluent, ill-defined lesions early in the disease course. This particular phenotype contributes considerably to misdiagnosis of a significant number of patients [76].

**6.2. Cerebrospinal Fluid (CSF) Analysis.** CSF provides valuable information about the inflammatory process of the CNS. Its analysis, which includes cellular profiles, oligoclonal bands, and IgG Index, has been used to define and differentiate MS from other disorders. The profile of the CSF in pediatric MS may vary depending on the child’s age. Compared to adolescents, children younger than 10 years of age tend to show more neutrophils in the CSF, and the CSF cellular profile in children tends to disappear in repeated analyses, on average 19 months after the initial examination [77]. The absence of neutrophils in the CSF at the onset of the disease may be a predictive factor of a second and early neurological episode. These observations suggest that the age of the patient exerts a modifying effect on the CSF cellular profile at the beginning of the disorder, which leads to activation of the innate immune system in the early stages or to an immature immune response [77].

CSF cell count and protein are normal in as many as 60% of pediatric patients with MS; the other patients show a discrete increase in the number of white blood cells or proteins [1, 78]. The percentage of pediatric patients with MS who also have oligoclonal bands has been reported to be up to 92%, providing that the spinal fluid is analyzed using isoelectric focusing assays [79, 80]. In some cases, the oligoclonal bands initially can be negative and detected only later in the course of the disease. It has been reported that positive oligoclonal bands may be found in 29% of patients with ADEM [78]. Mikaeloff et al., [55] in a study with 72 children presenting with a first demyelinating event, found

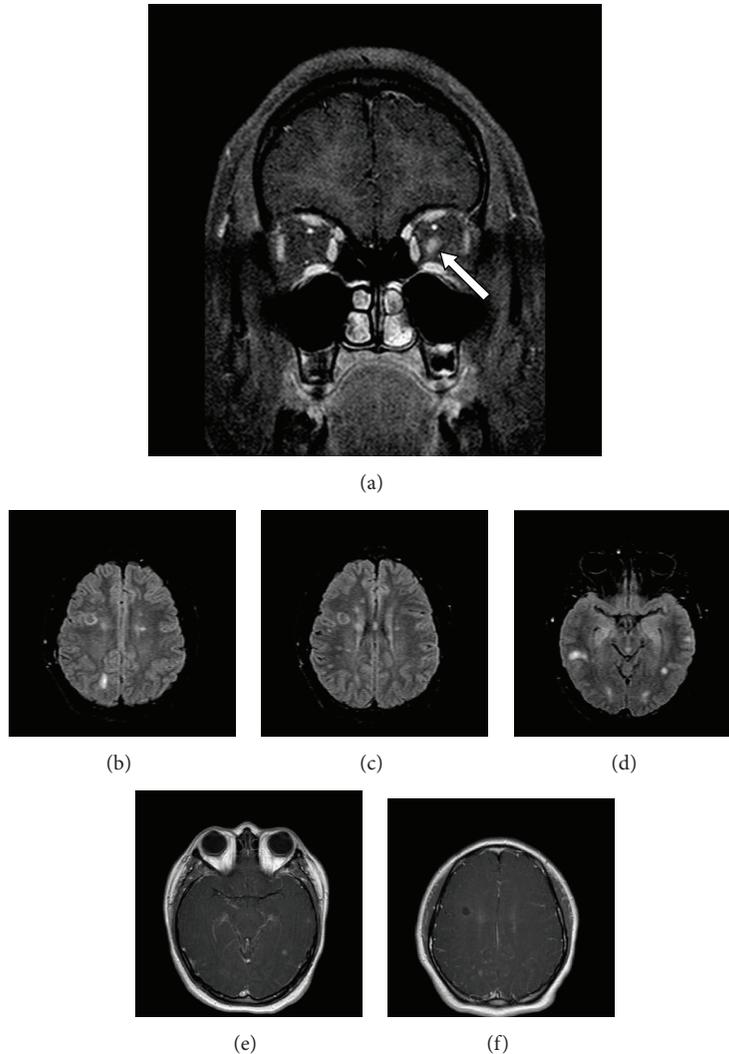


FIGURE 1: (a) Coronal T1 gadolinium enhanced sequence demonstrating left optic neuritis with enhancement and enlargement of the left optic nerve (arrow). (b) and (c) Axial FLAIR demonstrating typical well-circumscribed ovoid lesions in the juxtacortical and periventricular regions consistent with 2010 McDonald criteria for dissemination in space. (d) and (e) Axial FLAIR and gadolinium enhanced sequences with corresponding asymptomatic enhancing and nonenhancing lesions consistent with 2010 McDonald criteria for dissemination in time. (f) Axial T1 sequence with hypointense lesion associated with acute demyelination and axonal injury.

that 94% of children with positive oligoclonal bands went on to develop MS. Moreover, only 40% of patients with definitive diagnosis of MS had oligoclonal bands. These results suggest that oligoclonal bands have low sensitivity but high specificity for the development of MS.

**6.3. Visual Evaluation.** Visual deficit may go unnoticed in children with MS. Although ON may be a presenting symptom, a significant number of patients may have sub-clinical abnormalities of the visual pathway [81]. In fact, the visual pathways frequently are affected in MS, even in patients without visual disturbances. The visual evoked potential has diagnostic utility in pediatric MS, revealing a second focus of demyelination before a second clinical attack occurs [81]. Ocular coherence tomography (OCT), which permits *in vivo* characterization of the tissue structures with

higher resolution by quantifying the thickness of the retinal nerve fiber layer containing nonmyelinated axons as well as the macular volume, has been proposed as a useful tool to evaluate patients with demyelinating disorders [82, 83]. The determination of the total macular volume has been suggested as a marker for neuronal loss in patients with MS. Similarly, a correlation between reduction of retinal nerve fiber layer thickness and both brain atrophy (by MRI) and level of disability (by Kurtzke's EDSS score) also has been reported [84, 85]. In children with MS, this tool provides a sensitive demonstration of optic atrophy and, together with the ophthalmological assessment to include visual evoked potentials, provides objective evidence of a previous inflammatory insult to the optic nerve. A recent study on OCT in children reported a significant retinal atrophy in the pediatric population with demyelinating disorders including

optic neuritis, MS, and ADEM. Retinal atrophy was found to be more marked in patients with a previous episode of ON [86].

## 7. Treatment

**7.1. Acute Treatment.** Acute relapses of pediatric MS are usually treated with IV methyl prednisolone 20–30 mg/kg (maximum 1 g daily) for 3–5 days followed by oral taper. Available data in adults do not support the need for a corticosteroid taper after completion of pulse corticosteroid therapy. Pediatric patients with recurrent symptoms after discontinuation of intravenous corticosteroids may raise the possible need for an oral taper [87]. If there is an incomplete response or in case of a severe attack, intravenous immune globulin (IVIG) at 0.4 g/kg/day for 5 days or plasmapheresis should be considered.

**7.2. Preventive Therapy.** To date, there have been no randomized control trials of any DMT in the pediatric population, and the use of these treatments is mainly based on several adult clinical trials and small retrospective, observational studies. First-line therapies include intramuscular interferon (IFN)- $\beta$ 1a (300 mcg once a week), subcutaneous IFN  $\beta$ -1a (22 or 44 mcg 3 times a week), subcutaneous IFN- $\beta$ 1 b (0.25 mg every other day), or glatiramer acetate (20 mg/day) [88, 89].

Gradual titration of the interferon dosing over four to six weeks is common practice in children. In published studies, the majority of patients were escalated to full dose, unadjusted for age or body weight. Disease control is not always achieved immediately. Adherence to medication and time to effective dosing should be evaluated if relapses continue. If disease activity continues after 6–12 months of treatment, a change in therapies may be considered. Although there is no evidence-based guidelines as to when to switch therapies, working definitions of breakthrough disease in need of treatment modification from the IPMSSG suggest the following: (1) minimum time of full dose therapy of 6 months and (2) full medication adherence and one of the following: (a) increase or no reduction in the relapse rate or new T2 or enhancing lesion on MRI as compared to previous treatment or (b)  $\geq 2$  confirmed MRI or clinical relapses within a 12-month period [90].

Refractory disease may be considered if there are further relapses or silent progression of disease on MRI. There are several new immunomodulatory agents for refractory pediatric MS. These therapies include monoclonal antibody therapy (e.g., natalizumab, daclizumab), chemotherapeutic agents (e.g., cyclophosphamide, mitoxantrone), and oral medications with novel mechanisms of action (e.g., fingolimod, teriflunomide, and dimethyl fumarate (BG-12)). Among this group, only natalizumab, mitoxantrone, fingolimod, and teriflunomide have been approved by the FDA for use in adults with MS.

**7.3. Challenges regarding Current and Future MS Therapies.** Available data suggest that about 40% of pediatric MS patients discontinue treatment owing to intolerance, toxicity,

persisting relapses, or nonadherence, supporting a need for developing new therapies in this population. Only well-designed clinical trials and long-term safety monitoring may allow the pediatric patients to benefit from the advances in MS standard of care.

Recent legislation in the United States and Europe has now mandated pediatric studies for new biological products. In Europe, a pediatric investigation plan (PIP) must be submitted to the European Medicines Agency (EMA). Similarly, the Pediatric Research Equity Act (PREA) in the United States requires pediatric studies for any new active molecule, new dosage form, or new route of administration.

A full or partial waiver is possible if the treated condition does not occur in the pediatric population or if studies are not feasible or appropriate or safe for the age group. Additionally, the Best Pharmaceuticals Act for Children (BPCA) in the United States allows for voluntary pediatric drug assessments via written requests issued by the FDA, with the incentive of eligibility of an additional 6 months of market exclusivity [91].

A meeting report on Clinical Trial Summit from the Steering Committee of the International Pediatric MS Study Group (IPMSSG) has been recently published [91]. The academic leaders established guidelines for outcome measures, including clinical, cognitive, and MRI, to be considered in the pediatric MS clinical drug trials. Despite the growing arsenal of therapies that offers substantial promise for pediatric patients, there are some immediate and long-term health risks, and only well-designed, multicenter trials with long-term followup will properly assess accompanying hazards and safety.

## 8. Conclusions

The diagnosis of pediatric MS needs to be considered in those patients in whom optic nerve, sensory, motor, brainstem, and/or cerebellar disturbance are the presenting symptoms. A comprehensive history aided by clinical, neuroimaging, and laboratory clues can help to assure a prompt diagnosis and the exclusion of other neurological disorders. In younger patients, however, a polyfocal presentation with associated encephalopathy may be difficult to distinguish from ADEM. As in adult-onset MS, the MRI features of pediatric MS involve the presence of multiple lesions, mostly in the white matter and typically observed in the periventricular area of the corpus callosum and spinal cord. Children often show more infratentorial lesions, predominantly in the pons, and can have large and tumefactive lesions with perilesional edema. The most recent revision of the McDonald criteria specifically underscores its applicability in diagnosing MS in children older than 12 years and in facilitating the diagnosis at a first clinical attack, providing the criteria for dissemination in space and time are met.

During the last 10 years, new insights regarding the pathology and immunobiology, clinical features, and neuroimaging have increased the ability to better understand pediatric MS. For example, different studies have identified the potential roles of EBV and low vitamin D in the pathogenesis of MS. However, information about the nature

of the immune mechanisms involved in pediatric MS and the interactions of risk factors with genetic susceptibility is limited. On the horizon, identification of biomarkers with the promise to predict disease onset and monitor disease course, severity, and response to treatment has led to a renewed and increased interest and may provide important information for the best management of patients.

## References

- [1] P. Duquette, T. J. Murray, J. Pleines et al., "Multiple sclerosis in childhood: clinical profile in 125 patients," *Journal of Pediatrics*, vol. 111, no. 3, pp. 359–363, 1987.
- [2] A. Ghezzi, V. Deplano, J. Faroni et al., "Multiple sclerosis in childhood: clinical features of 149 cases," *Multiple Sclerosis*, vol. 3, no. 1, pp. 43–46, 1997.
- [3] M. Ruggieri, P. Iannetti, A. Polizzi, L. Pavone, and L. M. Grimaldi, "Multiple sclerosis in children under 10 years of age," *Neurological Sciences*, vol. 25, no. 4, pp. S326–S335, 2004.
- [4] L. B. Krupp, B. Banwell, and S. Tenenbaum, "International Pediatric MS Study Group, Consensus definitions proposed for pediatric multiple sclerosis and related disorders," *Neurology*, vol. 68, no. 16, supplement 2, pp. S7–S12, 2007.
- [5] L. B. Krupp, M. Tardieu, M. P. Amato et al., "International Pediatric Multiple Sclerosis Study Group criteria for pediatric multiple sclerosis and immune-mediated central nervous system demyelinating disorders: revisions to the 2007 definitions," *Multiple Sclerosis Journal*, vol. 19, no. 10, pp. 1261–1267, 2013.
- [6] O. Deryck, P. Ketelaer, and B. Dubois, "Clinical characteristics and long term prognosis in early onset multiple sclerosis," *Journal of Neurology*, vol. 253, no. 6, pp. 720–723, 2006.
- [7] C. Renoux, S. Vukusic, Y. Mikaeloff et al., "Natural history of multiple sclerosis with childhood onset," *New England Journal of Medicine*, vol. 356, no. 25, pp. 2603–2613, 2007.
- [8] A. Langer-Gould, J. L. Zhang, J. Chung, Y. Yeung, E. Waubant, and J. Yao, "Incidence of acquired CNS demyelinating syndromes in a multiethnic cohort of children," *Neurology*, vol. 77, no. 12, pp. 1143–1148, 2011.
- [9] B. Banwell, J. Kennedy, D. Sadovnick et al., "Incidence of acquired demyelination of the CNS in Canadian children," *Neurology*, vol. 72, no. 3, pp. 232–239, 2009.
- [10] I. A. Ketelslegers, C. E. Catsman-Berrepoets, R. F. Neuteboom et al., "Incidence of acquired demyelinating syndromes of the CNS in Dutch children: a nationwide study," *Journal of Neurology*, vol. 259, no. 9, pp. 1929–1935, 2012.
- [11] J. Kennedy, P. O'Connor, A. D. Sadovnick, M. Perara, I. Yee, and B. Banwell, "Age at onset of multiple sclerosis may be influenced by place of residence during childhood rather than ancestry," *Neuroepidemiology*, vol. 26, no. 3, pp. 162–167, 2006.
- [12] B. Banwell, A. Ghezzi, A. Bar-Or, Y. Mikaeloff, and M. Tardieu, "Multiple sclerosis in children: clinical diagnosis, therapeutic strategies, and future directions," *Lancet Neurology*, vol. 6, no. 10, pp. 887–902, 2007.
- [13] T. Chitnis, B. Glanz, S. Jaffin, and B. Healy, "Demographics of pediatric-onset multiple sclerosis in an MS center population from the Northeastern United States," *Multiple Sclerosis*, vol. 15, no. 5, pp. 627–631, 2009.
- [14] E. A. Yeh, T. Chitnis, L. Krupp et al., "Pediatric multiple sclerosis," *Nature Reviews Neurology*, vol. 5, no. 11, pp. 621–631, 2009.
- [15] T. Chitnis, L. Krupp, A. Yeh et al., "Pediatric Multiple Sclerosis," *Neurologic Clinics*, vol. 29, no. 2, pp. 481–505, 2011.
- [16] B. K. Mehta, "New hypotheses on sunlight and the geographic variability of multiple sclerosis prevalence," *Journal of the Neurological Sciences*, vol. 292, no. 1-2, pp. 5–10, 2010.
- [17] E. M. Mowry, "Vitamin D: evidence for its role as a prognostic factor in multiple sclerosis," *Journal of the Neurological Sciences*, vol. 311, no. 1-2, pp. 19–22, 2011.
- [18] S. Chen, G. P. Sims, X. C. Xiao, Y. G. Yue, S. Chen, and P. E. Lipsky, "Modulatory effects of 1,25-dihydroxyvitamin D3 on human B cell differentiation," *Journal of Immunology*, vol. 179, no. 3, pp. 1634–1647, 2007.
- [19] S. Simpson Jr., B. Taylor, L. Blizzard et al., "Higher 25-hydroxyvitamin D is associated with lower relapse risk in multiple sclerosis," *Annals of Neurology*, vol. 68, no. 2, pp. 193–203, 2010.
- [20] E. M. Mowry, L. B. Krupp, M. Milazzo et al., "Vitamin D status is associated with relapse rate in pediatric-onset multiple sclerosis," *Annals of Neurology*, vol. 67, no. 5, pp. 618–624, 2010.
- [21] E. M. Mowry, E. Waubant, C. E. McCulloch et al., "Vitamin D status predicts new brain magnetic resonance imaging activity in multiple sclerosis," *Annals of Neurology Journal*, vol. 72, no. 2, pp. 234–240, 2012.
- [22] A. Ascherio, K. L. Munger, E. T. Lennette et al., "Epstein-Barr virus antibodies and risk of multiple sclerosis: a prospective study," *Journal of the American Medical Association*, vol. 286, no. 24, pp. 3083–3088, 2001.
- [23] S. Alotaibi, J. Kennedy, R. Tellier, D. Stephens, and B. Banwell, "Epstein-Barr virus in pediatric multiple sclerosis," *Journal of the American Medical Association*, vol. 291, no. 15, pp. 1875–1879, 2004.
- [24] B. Banwell, L. Krupp, J. Kennedy et al., "Clinical features and viral serologies in children with multiple sclerosis: a multinational observational study," *Lancet Neurology*, vol. 6, no. 9, pp. 773–781, 2007.
- [25] D. Pohl, B. Krone, K. Rostasy et al., "High seroprevalence of Epstein-Barr virus in children with multiple sclerosis," *Neurology*, vol. 67, no. 11, pp. 2063–2065, 2006.
- [26] E. Waubant, E. M. Mowry, L. Krupp et al., "Common viruses associated with lower pediatric multiple sclerosis risk," *Neurology*, vol. 76, no. 23, pp. 1989–1995, 2011.
- [27] D. Pohl, "Epstein-Barr virus and multiple sclerosis," *Journal of the Neurological Sciences*, vol. 286, no. 1-2, pp. 62–64, 2009.
- [28] Y. Mikaeloff, G. Caridade, M. Rossier, S. Suissa, and M. Tardieu, "Hepatitis B vaccination and the risk of childhood-onset multiple sclerosis," *Archives of Pediatrics and Adolescent Medicine*, vol. 161, no. 12, pp. 1176–1182, 2007.
- [29] Y. Mikaeloff, G. Caridade, S. Assi, M. Tardieu, and S. Suissa, "Hepatitis B vaccine and risk of relapse after a first childhood episode of CNS inflammatory demyelination," *Brain*, vol. 130, part 4, pp. 1105–1110, 2007.
- [30] Y. Mikaeloff, G. Caridade, S. Suissa, and M. Tardieu, "Hepatitis B vaccine and the risk of CNS inflammatory demyelination in childhood," *Neurology*, vol. 72, no. 10, pp. 873–880, 2009.
- [31] Y. Mikaeloff, G. Caridade, M. Tardieu, and S. Suissa, "Parental smoking at home and the risk of childhood-onset multiple sclerosis in children," *Brain*, vol. 130, part 10, pp. 2589–2595, 2007.
- [32] D. A. Dymont, G. C. Ebers, and A. D. Sadovnick, "Genetics of multiple sclerosis," *Lancet Neurology*, vol. 3, no. 2, pp. 104–110, 2004.

- [33] P. L. de Jager, X. Jia, J. Wang et al., "Meta-analysis of genome scans and replication identify CD6, IRF8 and TNFRSF1A as new multiple sclerosis susceptibility loci," *Nature Genetics*, vol. 41, no. 7, pp. 776–782, 2009.
- [34] T. Derfuss, C. Linington, R. Hohlfeld, and E. Meinl, "Axo-glia antigens as targets in multiple sclerosis: implications for axonal and grey matter injury," *Journal of Molecular Medicine*, vol. 88, no. 8, pp. 753–761, 2010.
- [35] P. K. Stys, G. W. Zamponi, J. van Minnen, and J. J. Geurts, "Will the real multiple sclerosis please stand up?" *Nature Reviews Neuroscience*, vol. 13, no. 7, pp. 507–514, 2012.
- [36] K. Rostasy, E. Withut, D. Pohl et al., "Tau, phospho-tau, and S-100B in the cerebrospinal fluid of children with multiple sclerosis," *Journal of Child Neurology*, vol. 20, no. 10, pp. 822–825, 2005.
- [37] T. Derfuss, K. Parikh, S. Velhin et al., "Contactin-2/TAG-1-directed autoimmunity is identified in multiple sclerosis patients and mediates gray matter pathology in animals," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, no. 20, pp. 8302–8307, 2009.
- [38] E. K. Mathey, T. Derfuss, M. K. Storch et al., "Neurofascin as a novel target for autoantibody-mediated axonal injury," *Journal of Experimental Medicine*, vol. 204, no. 10, pp. 2363–2372, 2007.
- [39] E. Meinl, T. Derfuss, M. Krumbholz, A. K. Pröbstel, and R. Hohlfeld, "Humoral autoimmunity in multiple sclerosis," *Journal of the Neurological Sciences*, vol. 306, no. 1-2, pp. 180–182, 2011.
- [40] A. S. Dhaunchak, C. Becker, H. Schulman et al., "Implication of perturbed axoglial apparatus in early pediatric multiple sclerosis," *Annals of Neurology*, vol. 71, no. 5, pp. 601–613, 2012.
- [41] J. M. Van Noort, M. Bsibsi, W. H. Gerritsen et al., "αB-crystallin is a target for adaptive immune responses and a trigger of innate responses in preactive multiple sclerosis lesions," *Journal of Neuropathology and Experimental Neurology*, vol. 69, no. 7, pp. 694–703, 2010.
- [42] J. L. Bennett and G. P. Owens, "Cerebrospinal fluid proteomics: a new window for understanding human demyelinating disorders?" *Annals of Neurology*, vol. 71, no. 5, pp. 587–588, 2012.
- [43] C. H. Polman, S. C. Reingold, B. Banwell et al., "Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria," *Annals of Neurology*, vol. 69, no. 2, pp. 292–302, 2011.
- [44] R. F. Neuteboom, M. Boon, C. E. Catsman Berrevoets et al., "Prognostic factors after a first attack of inflammatory CNS demyelination in children," *Neurology*, vol. 71, no. 13, pp. 967–973, 2008.
- [45] M. Wilejto, M. Shroff, J. R. Buncic, J. Kennedy, C. Goia, and B. Banwell, "The clinical features, MRI findings, and outcome of optic neuritis in children," *Neurology*, vol. 67, no. 2, pp. 258–262, 2006.
- [46] L. H. Verhey, H. M. Branson, M. M. Shroff et al., "MRI parameters for prediction of multiple sclerosis diagnosis in children with acute CNS demyelination: a prospective national cohort study," *The Lancet Neurology*, vol. 10, no. 12, pp. 1065–1073, 2011.
- [47] S. Bigi and B. Banwell, "Pediatric multiple sclerosis," *Journal of Child Neurology*, vol. 27, no. 11, pp. 1378–1383, 2012.
- [48] G. Alper and L. Wang, "Demyelinating optic neuritis in children," *Journal of Child Neurology*, vol. 24, no. 1, pp. 45–48, 2009.
- [49] R. Miyazawa, Y. Ikeuchi, T. Tomomasa, H. Ushiku, T. Ogawa, and A. Morikawa, "Determinants of prognosis of acute transverse myelitis in children," *Pediatrics International*, vol. 45, no. 5, pp. 512–516, 2003.
- [50] A. T. Borchers and M. E. Gershwin, "Transverse myelitis," *Autoimmunity Reviews*, vol. 11, no. 3, pp. 231–248, 2012.
- [51] F. S. Pidcock, C. Krishnan, T. O. Crawford, C. F. Salorio, M. Trovato, and D. A. Kerr, "Acute transverse myelitis in childhood: center-based analysis of 47 cases," *Neurology*, vol. 68, no. 18, pp. 1474–1480, 2007.
- [52] D. Pohl and S. Tenenbaum, "Treatment of acute disseminated encephalomyelitis," *Current Treatment Options in Neurology*, vol. 14, no. 3, pp. 264–275, 2012.
- [53] S. Mar, J. Lenox, T. Benzinger, S. Brown, and M. Noetzel, "Long-term prognosis of pediatric patients with relapsing acute disseminated encephalomyelitis," *Journal of Child Neurology*, vol. 25, no. 6, pp. 681–688, 2010.
- [54] T. E. Lotze, J. L. Northrop, G. J. Hutton, B. Ross, J. S. Schiffman, and J. V. Hunter, "Spectrum of pediatric neuromyelitis optica," *Pediatrics*, vol. 122, no. 5, pp. e1039–e1047, 2008.
- [55] Y. Mikaeloff, S. Suissa, L. Vallée et al., "First episode of acute CNS inflammatory demyelination in childhood: prognostic factors for multiple sclerosis and disability," *Journal of Pediatrics*, vol. 144, no. 2, pp. 246–252, 2004.
- [56] D. J. A. Callen, M. M. Shroff, H. M. Branson et al., "Role of MRI in the differentiation of ADEM from MS in children," *Neurology*, vol. 72, no. 11, pp. 968–973, 2009.
- [57] D. M. Wingerchuk, "Neuromyelitis optica spectrum disorders," *CONTINUUM Lifelong Learning in Neurology*, vol. 16, no. 5, pp. 105–121, 2010.
- [58] A. McKeon, V. A. Lennon, T. Lotze et al., "CNS aquaporin-4 autoimmunity in children," *Neurology*, vol. 71, no. 2, pp. 93–100, 2008.
- [59] J. A. Peña, M. E. Ravelo, E. Mora-La Cruz, and C. Montiel-Nava, "NMO in pediatric patients: brain involvement and clinical expression," *Archivos de Neuro-Psiquiatria*, vol. 69, no. 1, pp. 34–38, 2011.
- [60] M. J. Morrow and D. Wingerchuk, "Neuromyelitis optica," *Journal of Neuro-Ophthalmology*, vol. 32, no. 2, pp. 154–166, 2012.
- [61] J. M. Tillema and A. McKeon, "The spectrum of neuromyelitis optica (NMO) in childhood," *Journal of Child Neurology*, vol. 27, no. 11, pp. 1437–1447, 2012.
- [62] B. Banwell, S. Tenenbaum, V. A. Lennon et al., "Neuromyelitis optica-IgG in childhood inflammatory demyelinating CNS disorders," *Neurology*, vol. 70, no. 5, pp. 344–352, 2008.
- [63] L. H. Verhey, E. D. van Pelt-Gravesteyn, A. Ketelslegers et al., "Validation of MRI predictors of multiple sclerosis diagnosis in children with acute CNS demyelination," *Multiple Sclerosis and Related Disorders*, vol. 2, no. 3, pp. 193–199, 2013.
- [64] M. P. Gorman, B. C. Healy, M. Polgar-Turcsanyi, and T. Chitnis, "Increased relapse rate in pediatric-onset compared with adult-onset multiple sclerosis," *Archives of Neurology*, vol. 66, no. 1, pp. 54–59, 2009.
- [65] L. Julian, D. Serafin, L. Charvet et al., "Cognitive impairment occurs in children and adolescents with multiple sclerosis: results from a United States network," *Journal of Child Neurology*, vol. 28, no. 1, pp. 102–107, 2013.
- [66] W. S. MacAllister, J. R. Boyd, N. J. Holland, M. C. Milazzo, and L. B. Krupp, "The psychosocial consequences of pediatric multiple sclerosis," *Neurology*, vol. 68, no. 16, supplement 2, pp. S66–S69, 2007.
- [67] W. S. MacAllister, C. Christodoulou, M. Milazzo, and L. B. Krupp, "Longitudinal neuropsychological assessment in pediatric multiple sclerosis," *Developmental Neuropsychology*, vol. 32, no. 2, pp. 625–644, 2007.

- [68] B. I. Glanz, B. C. Healy, L. E. Hviid, T. Chitnis, and H. L. Weiner, "Cognitive deterioration in patients with early multiple sclerosis: a 5-year study," *Journal of Neurology, Neurosurgery and Psychiatry*, vol. 83, no. 1, pp. 38–43, 2012.
- [69] J. O'Mahony, A. Bar-Or, D. L. Arnold, A. D. Sadovnick, R. A. Marrie, and B. Banwell, "Masquerades of acquired demyelination in children: experiences of a national demyelinating disease program," *Journal of Child Neurology*, vol. 28, no. 2, pp. 184–197, 2013.
- [70] J. S. Hahn, D. Pohl, M. Rensel, and S. Rao, "Differential diagnosis and evaluation in pediatric multiple sclerosis," *Neurology*, vol. 68, no. 16, supplement 2, pp. S13–S22, 2007.
- [71] M. Twilt and S. M. Benseler, "CNS vasculitis in children," *Multiple Sclerosis and Related Disorders*, vol. 2, no. 3, pp. 162–171, 2013.
- [72] L. C. McAdam, S. I. Blaser, and B. L. Banwell, "Pediatric tumefactive demyelination: case series and review of the literature," *Pediatric Neurology*, vol. 26, no. 1, pp. 18–25, 2002.
- [73] D. Riva, L. Chiapparini, B. Pollo, M. R. Balestrini, M. Massimino, and N. Milani, "A case of pediatric tumefactive demyelinating lesion misdiagnosed and treated as glioblastoma," *Journal of Child Neurology*, vol. 23, no. 8, pp. 944–947, 2008.
- [74] E. Waubant, D. Chabas, D. T. Okuda et al., "Difference in disease burden and activity in pediatric patients on brain magnetic resonance imaging at time of multiple sclerosis onset vs adults," *Archives of Neurology*, vol. 66, no. 8, pp. 967–971, 2009.
- [75] Y. Sadaka, L. H. Verhey, M. M. Shroff et al., "2010 McDonald criteria for diagnosing pediatric multiple sclerosis," *Annals of Neurology*, vol. 72, no. 2, pp. 211–223, 2012.
- [76] D. Chabas, T. Castillo-Trivino, E. M. Mowry, J. B. Strober, O. A. Glenn, and E. Waubant, "Vanishing MS T2-bright lesions before puberty: a distinct MRI phenotype?" *Neurology*, vol. 71, no. 14, pp. 1090–1093, 2008.
- [77] D. Chabas, J. Ness, A. Belman et al., "Younger children with MS have a distinct CSF inflammatory profile at disease onset," *Neurology*, vol. 74, no. 5, pp. 399–405, 2010.
- [78] R. C. Dale, C. de Sousa, W. K. Chong, T. C. S. Cox, B. Harding, and B. G. R. Neville, "Acute disseminated encephalomyelitis, multiphasic disseminated encephalomyelitis and multiple sclerosis in children," *Brain*, vol. 123, no. 12, pp. 2407–2422, 2000.
- [79] A. Ghezzi, C. Pozzilli, M. Liguori et al., "Prospective study of multiple sclerosis with early onset," *Multiple Sclerosis*, vol. 8, no. 2, pp. 115–118, 2002.
- [80] D. Pohl, K. Rostasy, H. Reiber, and F. Hanefeld, "CSF characteristics in early-onset multiple sclerosis," *Neurology*, vol. 63, no. 10, pp. 1966–1967, 2004.
- [81] D. Pohl, K. Rostasy, S. Treiber-Held, K. Brockmann, J. Gärtner, and F. Hanefeld, "Pediatric multiple sclerosis: detection of clinically silent lesions by multimodal evoked potentials," *Journal of Pediatrics*, vol. 149, no. 1, pp. 125–127, 2006.
- [82] F. Costello, S. Coupland, W. Hodge et al., "Quantifying axonal loss after optic neuritis with optical coherence tomography," *Annals of Neurology*, vol. 59, no. 6, pp. 963–969, 2006.
- [83] E. Frohman, F. Costello, R. Zivadinov et al., "Optical coherence tomography in multiple sclerosis," *Lancet Neurology*, vol. 5, no. 10, pp. 853–863, 2006.
- [84] E. Gordon-Lipkin, B. Chodkowski, D. S. Reich et al., "Retinal nerve fiber layer is associated with brain atrophy in multiple sclerosis," *Neurology*, vol. 69, no. 16, pp. 1603–1609, 2007.
- [85] A. P. D. Henderson, S. A. Trip, P. G. Schlottmann et al., "An investigation of the retinal nerve fibre layer in progressive multiple sclerosis using optical coherence tomography," *Brain*, vol. 131, part 1, pp. 277–287, 2008.
- [86] E. A. Yeh, B. Weinstock-Guttman, N. Lincoff et al., "Retinal nerve fiber thickness in inflammatory demyelinating diseases of childhood onset," *Multiple Sclerosis*, vol. 15, no. 7, pp. 802–810, 2009.
- [87] E. A. Yeh, "Management of children with multiple sclerosis," *Pediatric Drugs*, vol. 14, no. 3, pp. 165–177, 2012.
- [88] A. Ghezzi, M. P. Amato, M. Capobianco et al., "Disease-modifying drugs in childhood-juvenile multiple sclerosis: results of an Italian co-operative study," *Multiple Sclerosis*, vol. 11, no. 4, pp. 420–424, 2005.
- [89] E. A. Yeh, "Current therapeutic options in pediatric multiple sclerosis," *Current Treatment Options in Neurology*, vol. 13, no. 6, pp. 544–559, 2011.
- [90] T. Chitnis, S. Tenembaum, B. Banwell et al., "Consensus statement: evaluation of new and existing therapeutics for pediatric multiple sclerosis," *Multiple Sclerosis*, vol. 18, no. 1, pp. 116–127, 2012.
- [91] T. Chitnis, M. Tardieu, M. P. Amato et al., "International Pediatric MS Study Group Clinical Trials Summit: meeting report," *Neurology*, vol. 80, no. 12, pp. 1161–1168, 2013.