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REVIEW ARTICLE

Pharmacotherapy for Neuromyelitis Optica Spectrum Disorders: Current Management and Future Options

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Abstract

Neuromyelitis optica (NMO) is an inflammatory and demyelinating disease of the central nervous system. Although the prevalence of NMO is low, the rapid and severe impairment observed in patients has led to extensive development of research in the fields of diagnostic criteria and therapy in the past 15 years. With improved understanding of the pathophysiology of NMO and the role of aquaporin-4 (AQP4) or myelin oligodendrocyte glycoprotein antibodies, numerous therapeutic approaches have been proposed and are currently undergoing evaluation. In this review, we describe the rationale for existing therapeutics and their benefit/risk ratio. We also discuss the pharmacological and clinical interest of future approaches targeting, among others, B or T cells, the blood–central nervous system barrier, complement, polynuclear cells, AQP4-antibody linkage and AQP4 activity. The numerous agents under development are the result of a major collaborative effort all over the world. After the considerable progress on diagnosis, we are now close to class I evidence for a therapeutic effect of several drugs in NMO spectrum disorders, most notably with the anti-interleukin-6 receptor antibody (satralizumab) and anti-complement-5 antibody (eculizumab).

Key Points

The pathophysiology of neuromyelitis optica spectrum disorders (NMOSD) highlights the major role of adaptive immunity [with B cells and T cells (T-helper subtype 17) and aquaporin-4 (AQP4) or myelin oligodendrocyte glycoprotein (MOG) antibodies] and innate immunity, each representing a target for therapeutics.

Many drugs including monoclonal antibodies are under development, but the most advanced are inebilizumab (phase 2b), satralizumab (phase 3) and eculizumab (phase 3).

Modification of AQP4 protein expression in the central nervous system may be an innovative area of research, due to its role in brain inflammation, cytotoxic edema, and oxidative stress.

1 Pathogenesis and Clinical Symptoms of Neuromyelitis Optica (NMO)

Neuromyelitis optica (NMO) is an inflammatory and demyelinating disease of the central nervous system (CNS). Although the prevalence of NMO is low, the rapid and severe impairment observed in patients has led to extensive development of research in the fields of diagnostic criteria and therapy. Whereas historical descriptions showed a predominance of myelitis or optic neuritis in such patients, recent additional data have extended the phenotype of NMO, with magnetic resonance imaging (MRI) revealing the presence of clinically relevant lesions in the brain, diencephalon and brainstem [1, 2]. Contrary to multiple sclerosis (MS), disability in NMO is due to the consequences of the relapses, whereas progressive forms have only very rarely been reported [3, 4]. In 2004, the

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discovery of an aquaporin-4 (AQP4) antibody (AQP4-Ab) in the serum of NMO patients fundamentally modified our understanding of the pathology [5, 6]. This antibody is found in around 80% of NMO patients and has a proven pathogenic effect on AQP4, which is the main water channel of the CNS that is expressed on astrocytes. It is hypothesized that AQP4 peptides are recognized by T cells, mainly of the T-helper subtype 17 (Th17), and contribute to B-cell activation by conformationally intact AQP4 proteins. These B cells then differentiate into plasmablasts that secrete AQP4-Ab of the immunoglobulin G1 (IgG1) subtype. AQP4-Ab is thus able to cross the blood–CNS barrier and interact with AQP4 proteins expressed on astrocyte endfeet, leading to a decrease of AQP4 and glial fibrillary acidic protein (GFAP) expression, an astrocytic edema and the downregulation of excitatory amino acid transporter 2 (EAAT2), which impairs glutamate homeostasis [7–10]. Subsequent inflammation is characterized by an activation of the complement, by the classic pathway of C1q binding, produced locally by astrocytes, which in turn leads to an increase in blood–CNS barrier permeability and a massive infiltration of leukocytes, particularly eosinophils and neutrophils, which can be found in the cerebrospinal fluid (CSF) during the disease [11–13]. Complement-dependent cytotoxicity (CDC), antibody-dependent cell cytotoxicity (ADCC) and cellular infiltration results in dysfunction of astrocytes and, secondarily, astrocyte-dependent cells, such as oligodendrocytes and neurons [14].

Another antigenic candidate is myelin oligodendrocyte glycoprotein (MOG), a minor myelin component exclusively expressed on the surface of myelin sheaths in the CNS. The antibody against MOG (MOG-Ab) is found in 4–11% of NMO patients and does not co-occur with AQP4-Ab seropositivity. The prevalence of MOG-Ab at first presentation of acquired demyelinating syndromes is highly correlated with age, ranging from up to 50% of children to around 10% of adult patients [15]. To date, the level of proof for its involvement in NMO pathogenesis is lower than that for AQP4-Ab. Indeed, current knowledge is based on preclinical data showing that injection of human MOG-Abs induces an NMO-like syndrome in mouse models and complement-mediated demyelination in an ex vivo murine model [16–18]. In humans, histopathology data have shown reversible alterations to myelin without complement activation or inflammatory cell infiltration, as well as partial axonal preservation and reactive astrocyte scarring [19]. In CSF of NMO patients with MOG-Ab, an elevated MOG protein level without any detection of GFAP has been observed [20, 21]. Thus, MOG-Abs may drive a demyelination process without astrocytopathy, but it remains unclear whether MOG-Abs are primarily involved or if they are due to a bystander effect.

Fourteen to 22 percent of NMO patients have no detectable AQP4 or MOG antibodies. The pathophysiology of this double seronegative NMO spectrum disorder (NMOSD) population of patients is poorly documented. No evidence of astrocyte or oligodendrocyte alteration or CDC has been observed [14, 22]. Injection of seronegative patients' sera in a murine model failed to reproduce NMO-like lesions [23]. Due to a cellular response, particularly of the Th17 subtype, against AQP4, it is hypothesized that low titer antibodies against AQP4 may exist [24, 25]. Undiscovered autoantibodies could also exist in this population of patients. The clinical symptoms encountered in the different forms of NMOSD are summarized in Table 1.

2 Recent Developments in the Clinical Diagnosis of NMO

NMO was long considered to be a form of MS. Since the discovery of AQP4-Ab in the serum of NMO patients, this pathology is currently considered a distinct disease mediated primarily by B cells [5, 6]. AQP4-Ab is found in around 80% of NMO cases, and the recent diagnostic criteria revised in 2015 were built around AQP4-Ab as a key marker [37] (Table 2). NMOSD may currently be diagnosed even at a very early stage of the disease, and treatments are usually proposed after the first relapse. However, 20–30% of patients test negative for AQP4-Ab despite the clinical presentation being similar to that of AQP4-Ab-positive patients. Recently, a new antibody, MOG-Ab, was identified as a second NMOSD marker [31]. MOG-Ab has not been included in the new set of criteria, except in younger patients, but the spectrum of the disease seems to be quite similar. In children, MOG-Abs may be found in acute demyelinating encephalomyelitis (ADEM) or autoimmune encephalitis and are sometimes transient [38]. It is usually recommended that patients are retested 3–6 months later to verify if the antibodies are still present; if they are not, the neurological disease may be monophasic, and this finding may have some importance regarding the therapeutic strategy.

3 Current Treatment Strategies with Immunosuppressants

3.1 Treatment of Relapses

Due to the possibility of severe residual disability following relapses, the management of acute exacerbation is of paramount importance and needs to be started early. Patients are usually treated with 1 g of intravenous (IV) methylprednisolone (IVMP) for 3–5 days. Relapses that do not respond to IV steroids may benefit from five to seven plasma exchange (PLEX) procedures over a 2-week

Table 1 Symptoms of neuromyelitis optica spectrum disorders (NMOSD) in adult patients stratified by serological status [26–36]

	Serological status of patients fulfilling the NMOSD 2015 criteria (prevalence, %)		
	NMOSD with AQP4-Ab (80%)	NMOSD with MOG-Ab (4–11%)	NMOSD with neither MOG-Ab nor AQP4-Ab (14–22%)
Ratio F:M	10:1	1:1	1:1
Mean age at onset, years	40	30	40
Characteristic of the first attack	Longitudinally extensive transverse myelitis or optic neuritis in around 40% Trend for more myelitis with increasing age at onset	Mainly optic neuritis in around 30%, often unilateral, with optic disc swelling and severe visual impairment at nadir	Mainly bilateral optic neuritis or simultaneous optic neuritis and longitudinally extensive transverse myelitis
Clinical course	Relapsing Overrepresentation of myelitis Brain, brainstem or cerebellum manifestations corresponding to AQP4 location in the CNS	Relapsing Overrepresentation of optic neuritis Less frequent brain, brainstem or cerebellum manifestations than AQP4-Ab patients	Mainly relapsing or monophasic in around 25%
Disability	Poor motor and visual recovery	Better recovery than AQP4-Ab patients	Poor motor and visual recovery

Ab antibody, *AQP4* aquaporin-4, *CNS* central nervous system, *F* female, *M* male, *MOG* myelin oligodendrocyte glycoprotein

Table 2 2015 criteria for neuromyelitis optica spectrum disorders (NMOSD) [37]

NMOSD with AQP4 antibodies	NMOSD without AQP4 antibodies or serological status unknown
At least one core clinical manifestation of NMOSD (see below)	At least two different clinical manifestations of NMOSD At least one optic neuritis or myelitis or area postrema syndrome MRI in accordance with core clinical manifestations
Core clinical manifestations of NMOSD:	
Optic neuritis (extension of optic neuritis should be more than the half or the optic nerve or include the optic chiasm. A normal or non-specific brain MRI is also accepted)	
Acute myelitis (extension of myelitis in the sagittal plane should be more than three vertebral segments. An extensive atrophy of the spinal cord is also accepted in a patient with a history of myelitis)	
Area postrema syndrome	
Acute brainstem syndrome (should include at least one periependymal brainstem lesion)	
Symptomatic narcolepsy	
Symptomatic brain syndrome (ADEM/PRES)	

These criteria are applicable only in cases of no better clinical explanation

Recommendation: AQP4 should be tested with a cell-based assay

ADEM acute demyelinating encephalomyelitis, *AQP4* aquaporin-4, *MRI* magnetic resonance imaging, *PRES* posterior reversible encephalopathy syndrome

period [39]. A recent study demonstrated the major impact of PLEX, particularly during acute myelitis [40], and the need to start this therapeutic strategy early after a relapse [41]. As the diagnosis of NMOSD cannot be made immediately without the results of AQP4 or MOG antibody testing, it could be argued that the first episode of a suspected NMOSD clinical manifestation should be managed with the same procedure. Once NMOSD is confirmed, additional immunosuppression should be considered. Oral prednisone (1 mg/kg) for 1–6 months can be initiated after IVMP or PLEX to ensure a prolonged effect on inflammation until steroid-sparing immunosuppressants take effect [42]. Only one report assessed the efficacy of intravenous immunoglobulin (IVIG) at a dose of 2 g/kg body weight for acute exacerbation of NMO, but that study had some limitations due to the small size of the cohort (ten patients) [43]. To date, no differences have been clearly demonstrated in the therapeutic response to IVMP or PLEX in NMOSD patients according to their serological status. Some observational studies have shown that

MOG-Ab-associated diseases are highly steroid responsive at relapse, while some patients can exhibit symptom flare-ups after withdrawal or tapering of steroids [32, 44].

3.2 Maintenance Therapy

3.2.1 Rituximab

The US Food and Drug Administration (FDA) initially approved rituximab (RTX) for the treatment of non-Hodgkin B-cell lymphomas in 1997. RTX is a chimeric monoclonal antibody (mAb) against human CD20, made with a variable light chain of murine anti-CD20 and a constant heavy chain (Fc) of human IgG1 associated with a light chain Kappa. CD20 is expressed at the membrane of the B lymphocyte from the stage of pre-B cells to mature B lymphocyte and could possibly play a role in cytosolic calcium flux [45]. Interestingly, CD20 is also expressed in less than 5% of T lymphocytes [46]. RTX was classified as a type I mAb [47] and acts through the relocalization of CD20 into lipid raft with minimal direct cell death [48]. Its major mechanism of action is the result of destruction of B cells caused by antibody-dependent phagocytosis (ADP) by macrophages and neutrophils, CDC or ADCC involving natural killer (NK) cells. These mechanisms depend on the Fc portion of the antibody binding to the Fc gamma receptors (FcγRs) on immune cells, with a potential low affinity of certain polymorphisms of the FcγR3A, including the FcγR3A-158F allele, as shown in NMO [49]. Studies on pharmacokinetics show that RTX infused intravenously has a terminal half-life of about 120 h and can persist in the body for up to 6–9 months after treatment stops [50]. A weak diffusion in the CNS has been observed because RTX cannot cross the blood–CNS barrier. After IV administration, maximal RTX levels in CSF are generally less than 1% of serum levels [51, 52]. One study shows that body mass index's effect on drug disposition and its consequent impact on the effective dose could be associated with RTX response [53]. There is general agreement that the induction phase should be based on the infusion of about 2 g during 1 month, consisting of either two infusions of 1 g 2 weeks apart or 375 mg/m^2 every week for 4 consecutive weeks. Classical maintenance therapy is either two infusions of 1 g 2 weeks apart, one infusion of 1 g, one infusion of 375 mg/m^2, or, as proposed in Chinese patients, a low dose of RTX without increasing the risk of disease reactivation [54, 55]. RTX depletes B cells from the circulation within 1 month after administration. The degree of B-cell depletion is variable among patients, but restoration of the B-cell repertoire generally takes 9–12 months from the last perfusion of RTX [56]. In the setting of a disrupted blood–CNS barrier, depletion occurs not only in the periphery, but also in the perivascular area in the brain parenchyma [57].

Despite RTX being widely used in NMO, the available data on RTX efficacy are derived only from open-label, uncontrolled and non-randomized observational studies. A meta-analysis including 25 studies with more than two patients and explicit data on efficacy found a reduction in the mean annualized relapse rate (ARR) of 0.79 [95% confidence interval (CI) − 1.09 to − 0.5] and in the mean Expanded Disability Status Scale (EDSS) score of 0.64 (95% CI − 1.18 to − 0.1) after a mean 27.5 months in a population of patients positive for AQP4-Ab in around 80% of cases [58]. Additional data show that the percentage of patients who are relapse free after 12–60 months of RTX therapy ranges from 33 to 100% [49, 53, 54, 59–66]. In view of its strong efficacy and the disabling potential of NMO, this drug is increasingly used as a first-line therapy [66–68]. This success in clinical practice is supported by the safety data, showing an adverse event rate of only 26%, including mainly infusion-related adverse effects (10%), infection (9%) or persistent leukopenia (4%) [58]. No progressive multifocal leukoencephalopathy (PML) has been described with RTX in NMO. Deaths have been reported in 1.6% of patients receiving RTX, but the causes were not clearly identified.

3.2.2 Mycophenolate Mofetil

Mycophenolate mofetil (MMF) has been used since the 1980s for its immunosuppressive property in the prevention or treatment of acute rejection in organ transplantation and is currently used "off-label" in autoimmune diseases such as NMO. This compound is a semi-synthetic derivative of mycophenolic acid (MPA), which is the active metabolite of MMF. MPA acts as a selective non-competitive inhibitor of inosine 5-monophosphate dehydrogenase type II, which is a rate-limiting enzyme in the de novo synthesis of guanine ribo- and 2-deoxyribonucleotides. MPA has a mean terminal half-life of 17 h and has been shown to prevent the production of interferon gamma (INF-γ), lipopolysaccharide-induced interleukin-6 (IL-6) and oxidative stress [69]. At a cellular level, MPA depletes the guanosine pool in lymphocytes and inhibits T- and B-cell proliferation/transendothelial migration, macrophage activation, dendritic cell function and immunoglobulin production [70]. It is generally agreed that the MMF target dosage should range between 1500 and 3000 mg/day [71], resulting in a total lymphocyte count of around 1000–1500/μL. Unfortunately, few studies have reported the lymphocyte count or the area under the curve for MMF concentration in plasma, leading to speculation about the pharmacological effect of the drug.

Other biases in the published studies are their retrospective design, the inclusion of mainly AQP4-Ab-positive patients and the mixed use of MMF as first-line or rescue therapy. One study distinguished patients by their serotype (45 AQP4-Ab seropositive, 5 MOG-Ab seropositive, 17 double seronegative) and assessed MMF as a first-line therapy at a fixed dosage of 2000 mg/day [42]. In this study, MMF resulted in a strong reduction of the ARR from 1 to 0 after a median follow-up of 24 months, 49% of the patients remaining relapse free. The EDSS score improved or stabilized in 80% of patients. No difference in terms of efficacy and safety was observed according to the serological status, a finding that was subsequently confirmed by another study using MMF as first-line therapy [72]. Concomitant use of oral steroids during 2–3 months after MMF initiation could prevent an early relapse in view of the delay before MMF has a pharmacological effect. Previous studies reported a similar range of efficacy in terms of ARR or EDSS, with 15–25% of adverse events due to digestive intolerance and non-fatal infection [73–76].

3.2.3 Azathioprine

Azathioprine (AZA) is a prodrug form of 6-mercaptopurine (6-MP) which was first introduced in clinical practice in the 1960s for kidney transplantation to prevent immunological rejection. AZA is converted non-enzymatically to 6-MP, which is metabolized in the liver to the active metabolite 6-thioinosinic acid and works as a purine antagonist that gives negative feedback on purine metabolism and inhibits DNA and RNA synthesis. Its action results in the inhibition of T-cell activation, a reduction in antibody production and a decrease in the level of circulating monocytes and granulocytes. 6-MP has a terminal half-life of between 0.5 and 2 h [77]. AZA is widely used to treat a variety of autoimmune diseases, including NMO.

The first small open-label case series reported strong efficacy of AZA at 2 mg/kg/day in association with oral corticotherapy, with an improvement in median EDSS score and a marked decline in ARR [78, 79]. Most substantial retrospective studies have confirmed these preliminary results, showing in more than 200 AQP4-Ab-positive patients, a decrease in ARR in more than 70%, with an increasing effect over time, the ARR being higher in the first 12 months of treatment [76, 80, 81]. In these studies, no differences were observed in terms of efficacy according to the antibody status of the patients. A long-term evaluation of 100 patients under AZA therapy at 3–4 mg/kg/day in addition to 1 mg/kg/day of prednisone during 3–6 months showed a nearly 80% chance reduction in the hazard of EDSS worsening after 5 years and no severe side effects after a median of 8 years [82]. Side effects requiring AZA interruption in this long-term study were gastrointestinal intolerance in 4%, severe infection or liver toxicity in 2% and, more rarely, alopecia or allergy/skin reaction. Despite these interesting results, AZA remains the most challenged immunosuppressive treatment in NMO in view of the fact that corticotherapy should be maintained for 3–6 months while awaiting the pharmacological effect of AZA. Furthermore, two retrospective studies and one open-label, underpowered, randomized clinical trial comparing AZA to MMF and RTX suggested that AZA could be less effective than MMF or RTX in terms of ARR [75, 76, 83]. According to the design of these studies, these results should be interpreted with caution in view of the various biases relating to patient selection, the dosage used and follow-up.

3.2.4 Mitoxantrone

Mitoxantrone (MITO) is an anti-neoplastic anthracenedione derivative related to the anthracyclins doxorubicin and daunorubicin. MITO inhibits B-cell functions, including antibody secretion, abates helper and cytotoxic T-cell activity, and decreases the secretion of Th1 cytokines, such as INF-γ, tumor necrosis factor (TNF), and IL-2 [84]. In NMO, a prospective, open-label, 2-year study reported a clinical and MRI improvement in four out of five patients treated with MITO [85]. In a study that evaluated the quantitative modification of AQP4-Ab blood level in patients treated with several immunosuppressive therapies, three patients were treated with MITO: one patient experienced a strong improvement in relapse rate and a decrease in AQP4-Ab blood level, whereas the other two patients were unresponsive regarding these two evaluation criteria [79]. However, patients in this study were treated with several immunosuppressive therapies and the dose and protocol of each treatment was not reported, thus making the results difficult to interpret.

3.2.5 Cyclophosphamide

Cyclophosphamide (CYC) is a prodrug converted by the liver to active alkylating metabolites, which bind to a guanine base of DNA and interfere with mitosis. Treatment with CYC causes suppression of cell-mediated and humoral immunity through its effects on B and T cells. It decreases the secretion of INF-γ and IL-12 by monocytes and increases secretion of IL-4 and IL-10 from peripheral blood mononuclear cells (PBMCs). Furthermore, this drug selectively targets CD45/CD4/RA$^+$ T cells and increases the number of Th2 cells [86]. CYC has a terminal half-life of between 3 and 12 h, and the immune system returns to baseline 3–12 months after cessation of the treatment [87]. Studies relating experience in treating NMO with CYC are mainly case reports of patients who had an association with NMO and another autoimmune systemic disease, such as

systemic lupus erythematosus [88–90] or Sjögren's syndrome [91]. A demonstrative case report shows that the use of CYC can be successful after an unresponsive experience with high-dose corticotherapy, IVIG, MMF, tacrolimus, low-dose daily oral CYC and RTX [90]. In a patient without any systemic disease, the oral long-term therapy (50 mg daily) was associated with a decrease in AQP4-Ab level and a strong reduction in relapse rate (2.82/year to 0.23/year) during a 4.4-year follow-up [79].

4 Recent Studies on Agents Undergoing Clinical Development for NMO

4.1 Phase 2 and 3 (i.e., Monoclonal Antibodies)

4.1.1 Inebilizumab (MEDI-551)

Inebilizumab (MEDI-551) is a humanized mAb of IgG1 kappa subtype directed against the extracellular loop of CD19 B-cell protein. The anti-CD19 mAb was therefore produced in an α1,6-fucosyltransferase-deficient Chinese hamster ovary (CHO) cell line to generate fucose-free anti-CD19 mAb. This condition enables MEDI-551 to bind more tightly to FcγRIIIa, an activating IgG receptor expressed on NK cells and on macrophages that mediate ADCC [92]. After binding to CD19 on B cells, MEDI-551 generates a cytotoxic T-lymphocyte response and a strong ADCC phenomenon that suppresses B cells from pro-B cells to early plasmablasts. Compared with RTX, MEDI-551 may potentially provide a broader depletion along the B-cell lineage and could remove the plasmablasts that are producing AQP4-Abs or MOG-Abs.

Although there are as yet no reports of MEDI-551 having been used in NMOSD, a phase 1 study in relapsing MS showed it had an acceptable safety profile and a possible action on gadolinium-enhancing lesions on MRI [93]. To date, MEDI-551 has entered a multicenter, randomized, double-blind, placebo-controlled, phase 2b trial in NMOSD (clinical trial.gov identifier: NCT02200770).

4.1.2 Satralizumab

Satralizumab (SA237), like tocilizumab, is a humanized recombinant mAb targeting the IL-6 receptor (IL-6R) with immunomodulatory potential. IL-6 is a pro-inflammatory pleiotropic cytokine produced by a large number of cell types, including T and B cells, monocytes and fibroblasts. It participates in various physiological processes, such as T-cell activation, induction of immunoglobulin secretion, and induction of hepatic protein synthesis in the acute phase of inflammation. In NMO, IL-6 promotes the differentiation of inflammatory Th17 cells and plasmablasts, inducing production of pathogenic antibodies. It also increases blood–CNS barrier permeability, allowing infiltration of antibodies and pro-inflammatory cells into the CNS. Tocilizumab specifically binds to soluble and membrane receptors for IL-6 and inhibits their signal transmission. A few case reports or short series on treatment with tocilizumab in NMO have been published. The initial results were quite encouraging even in patients who were resistant to other drugs [94–96]. Recently, the humanized IgG2 satralizumab was designed to improve pharmacokinetics by applying the "antibody recycling technology" to tocilizumab [97]. This technology leads to increased dissociation of tocilizumab from IL-6R within the acidic environment of the endosome (pH 6.0), while maintaining its binding affinity to IL-6R in plasma (pH 7.4). This pH-dependent IL-6R dissociation in the endosome resulted in lysosomal degradation of the previously bound IL-6R, while releasing the free antibody back to the plasma to bind another IL-6R molecule.

Two phase III studies have been conducted with satralizumab. The objective of these studies was to evaluate the efficacy, safety, pharmacodynamic, pharmacokinetic and immunogenic profiles of SA237 in patients with NMOSD (clinical trial.gov identifiers: NCT02073279 and NCT02028884). The first study evaluated SA237 against placebo and the second was an "add on" study with oral immunosuppressive drugs (AZA or MMF). The results of the "add on" study were presented at the European Committee for Treatment and Research in Multiple Sclerosis (ECTRIMS) congress in Berlin 2018 [98]. The reduction in risk of relapse in the treated group was 62% ($p = 0.018$) and increased to 79% if one considers only the AQP4-Ab-positive group. For the placebo control study, recruitment has been completed and the results will be available in 2019.

4.1.3 Eculizumab

Eculizumab is a humanized mAb that binds with high affinity to terminal complement component C5 and prevents its cleavage to the prothrombotic, pro-inflammatory analphylatoxin C5a and the prothrombotic, pro-inflammatory, cytolytic C5b-9 complex. The constant region of the antibody neither activates complement nor binds to Fc receptors of cells, and avoids an off-target activity. The onset of action of eculizumab can occur 10 min after dosing and by 1 h after first dose. This drug is currently approved in the USA and Europe for paroxysmal nocturnal hemoglobinuria, atypical hemolytic uremic syndrome and myasthenia gravis. In NMO, a first open-label study in 14 patients seropositive for AQP4-Ab demonstrated that eculizumab reduced the rate of attacks in aggressive disease, supporting the role of complement activation in the pathogenesis of NMO [99]. Recently, a double-blind, randomized study comparing eculizumab and placebo as an add-on therapy to AZA or MMF in 143

AQP4-Ab-positive NMOSD patients has been performed (NCT01892345). Despite the lack of scientific validation upon an external peer review committee, Alexion Pharmaceuticals reported that the study met the primary endpoint of the time to first on-trial relapse, demonstrating that eculizumab treatment reduces the risk of relapse of 94.2% in NMOSD compared to placebo. At 48 weeks, 97.9% of patients receiving eculizumab were relapse free, compared to 63.2% of patients receiving placebo. In this study, eculizumab was generally well tolerated, with a safety profile consistent with that observed in previous clinical studies and actual use in the three approved indications. In particular, the use of meningococcal vaccination before randomization prevented effectively the known risk of meningococcal infection. Eculizumab has recently received orphan drug designation from the FDA and the European Medicines Agency (EMA) for the treatment of NMOSD. The final presentation of the results should be presented to the American Academy of Neurology in 2019. Interestingly, the "antibody recycling technology," applied to tocilizumab, is being developed with eculizumab to improve the treatment's antigen-neutralizing ability and provide pharmacokinetics suitable for less frequent administration [100].

4.2 Phase 1 and Preclinical Studies

The pathophysiological hypothesis for NMOSD with AQP4-Ab is based on the peripheral production of AQP4-IgG, which can cross the blood–CNS barrier because of an increased permeability and bind to AQP4 on perivascular astrocyte endfeet. This binding induces activation of the complement, with an inflammatory response (cytokine production recruits eosinophils and neutrophils, corresponding to the time of cell infiltration). Following the degranulation of neutrophils, astrocytes are damaged first, followed by oligodendrocyte lesions which cause axonal degeneration and neuronal death. Mature lesions are characterized by pan-necrosis and widespread infiltration by macrophages. Future preventive therapies will need to target immune cell proliferation or activation, circulating AQP4-Ig, blood–CNS barrier permeability, complement activation and infiltration of leukocytes (Fig. 1).

4.2.1 Targeting CD20 B Cells

After maturation into antibody-producing plasma cells and by producing antibodies against AQP4, B cells and humoral immune system dysregulation appear to be the cornerstone of NMO. In addition to RTX, another chimeric mAb targeting CD20, namely ublituximab (LFB-R603, TGT-1101, TGTX-1101), has been developed more recently. This glycoengineered antibody contains low fucose, leading to improved FcγRIIIa binding and enhanced ADCC compared to RTX. Ublituximab is currently being evaluated in phase 3 studies for patients with chronic lymphocytic leukemia [101]. Its clinical development is focused on cancer and in MS. Two randomized, multicenter, double-blinded, phase 3 studies (ULTIMATE I and ULTIMATE IADCC) are currently recruiting patients with relapsing forms of MS. Whereas RTX is widely used at present as a first-line NMO treatment, a single-center, open-label, phase 1 study is in progress to assess the safety of ublituximab as an add-on therapy to corticosteroids for the treatment of acute optic neuritis and/or transverse myelitis in NMO and NMOSD (Clinical trial.gov identifier: NCT02276963). By increasing ADCC activity, ublituximab dosage could be reduced and adverse events, such as reactivation of hepatitis B virus infection, could be decreased compared to RTX. The study was completed in July 2018.

4.2.2 Targeting CD19 B Cells

The depletion of CD19-expressing cells appears to be an interesting strategy. It could be efficacious, but carries a potentially high risk of infectious complications. Targeting CD19 instead of CD20 during B-cell maturation might severely affect antibody-secreting plasma cells in the bone marrow, therefore resetting the immune repertoire of antibody-secreting plasma cells. Several recombinant CD19 mAbs are currently under development. The most advanced compound is MEDI-551, as described above, but another therapeutic approach, using the tandem chimeric antigen receptor (CAR) T-cell therapy, based on the reduction of the outgrowth of antigen escape variants by targeting two antigens simultaneously [102], is currently being explored in NMO after having being used in systemic lupus erythematosus. This phase 1, open-label, safety and proof-of-concept trial is assessing the safety and efficacy of tandem chimeric antigen receptor T cells transduced with the anti-CD19/CD20 vector (tanCART-19/20) cells at three doses ($1–2\times10^5$, $3–6\times10^5$ or $1–2\times10^6$ CAR-T cells/kg) in NMOSD patients having received a 12-day long pretreatment with high IV doses of methylprednisolone (Clinical trial.gov identifier: NCT03605238). After the presumed recruitment of nine patients, the results are expected in the middle of August 2020.

4.2.3 Inhibiting the Binding of Circulating Aquaporin-4 (AQP4) Antibody to AQP4

Recently, a new strategy has emerged. Its goal is to inhibit the interaction between AQP4 and the pathogenic AQP4-Ab. Aquaporumab is a recombinant human mAb which, instead of trapping AQP4-Ab in the blood and CSF, crosses the

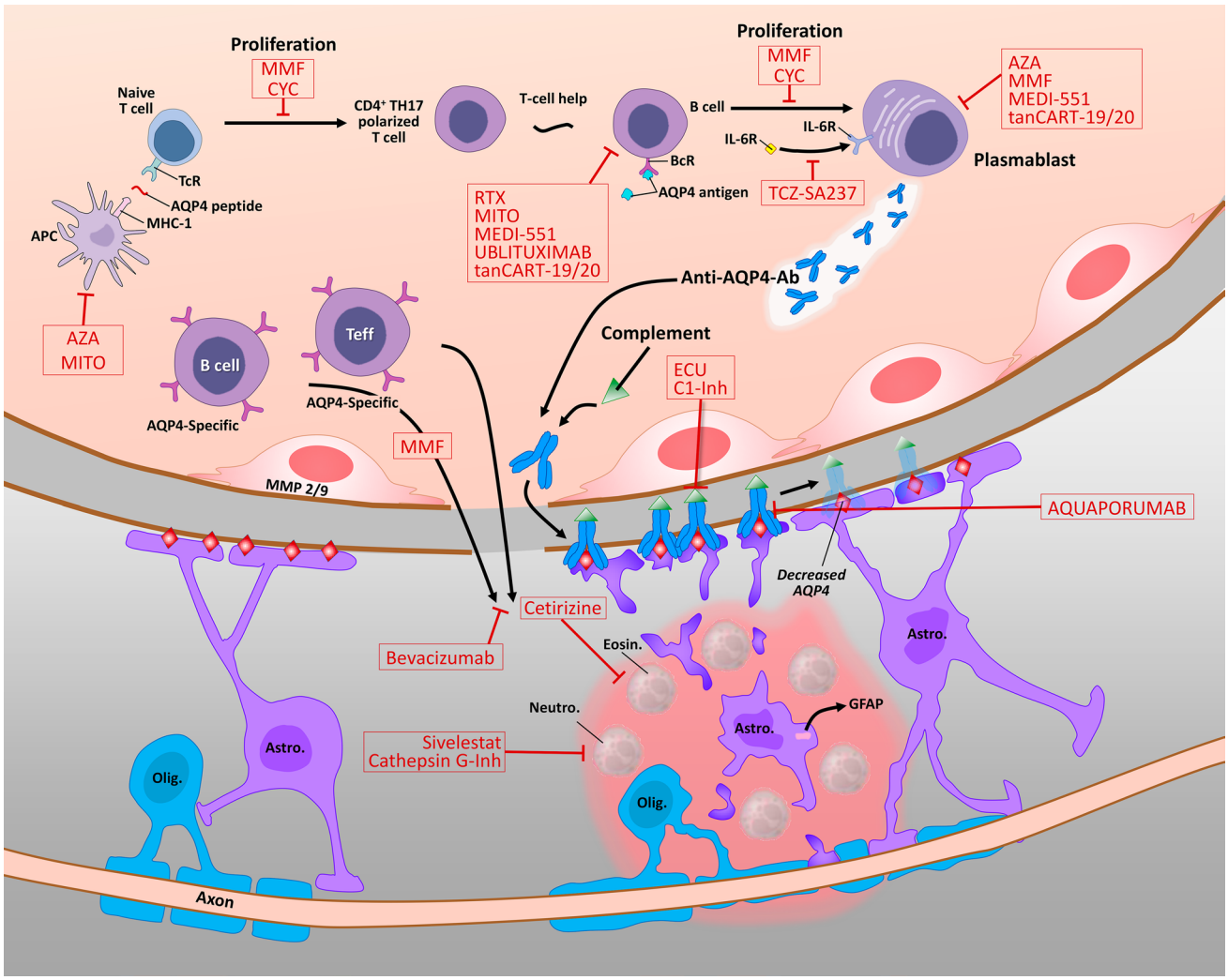

Fig. 1 Pharmacological effects of the drugs currently used or assessed in neuromyelitis optica spectrum disorders, especially when AQP4 antibodies are presents. *Ab* antibody, *APC* antigen-presenting cell, *AQP4* aquaporin-4, *Astro* astrocyte, *AZA* azathioprine, *BcR* B-cell receptor, *Cathepsin G-Inh* Cathepsin G-Inhibitor, *CYC* cyclophosphamide, *C1-Inh* C1-esterase inhibitor, *ECU* Eculizumab, *Eosin* Eosinophil, *GFAP* glial fibrillary acidic protein, *IL-6R* interleukin-6 receptor, *MEDI-551* inebilizumab, *MHC-1* major histocompatibility complex class 1, *MITO* mitoxantrone, *MMF* mycophenolate mofetil, *MMP* matrix metalloproteinases, *Neutro* Neutrophil, *Olig* oligodendrocyte, *RTX* rituximab, *SA237* satralizumab, *tanCART-19/20* tandem chimeric antigen receptor T cells transduced with the anti-CD19/CD20 vector, *TCR* T-cell receptor, *TCZ* tocilizumab, *TH* T helper

blood–brain barrier and then binds to AQP4 by means of its Fab moiety [103]. This association prevents the binding of the pathogenic antibody, but, in contrast to the AQP4-Ab, the mutated Fcγ portion does not activate the antibody-dependent complement and cell-dependent mediated cytotoxicity. Its competitive inhibition of AQP4-Ab binding depends on the greater affinity of aquaporumab, compared to that of pathological AQP4-Ab, to the AQP4 protein. Its safety will depend on its selectivity for the different AQP proteins and the lack of functional effect on AQP4 itself. The effects of this molecule have been tested in vitro and in vivo [104]. In cell cultures, aquaporumab effectively prevents complement- and cell-mediated cytotoxicity from NMO patients' sera. In in vivo NMO mouse models, aquaporumab prevents brain lesions and demyelination 24 h after treatment. This attractive approach is now to be confirmed in clinical studies.

At last, high-throughput screening of ~60,000 compounds has identified small molecules such as the antiviral Arbidol, the flavonoid tamarixetin, and several plant-derived berbamine alkaloids that competitively inhibit pathogenic AQP4-IgG binding, thereby reducing by more than 80% the severity of NMO lesions in an ex vivo spinal cord slice culture model of NMO and in mice in vivo [105].

4.2.4 Targeting Blood–CNS Barrier Permeability

As discussed in the introduction, maintaining the integrity of the blood–CNS barrier appears to be a crucial endpoint

in NMO. Indeed, restoring the structure and function of the blood–CNS barrier should limit the crossing of AQP4-IgG to brain astrocytes. Moreover, in in vitro studies, NMO-IgG binding to astrocytes further increases permeability in a human endothelium/astrocyte barrier model [106]. More precisely, the expression of tight junction proteins is reduced by the incubation of human brain microvascular endothelial cells with serum from AQP4-IgG-seropositive patients. This alteration of the barrier is reversed by pre-treatment with anti-vascular endothelial growth factor (anti-VEGF) neutralizing antibodies [107]. These data suggest the involvement of autocrine or paracrine secretion of VEGF by astrocytes or endothelial cells, but the mechanism linking AQP4-IgG and VEGF secretion is not clear. Whatever the pathological mechanism involved in increased blood–CNS barrier permeability, a proof-of-concept trial, namely a phase 1b, single-center and open-label study, was conducted to evaluate the safety and efficacy of bevacizumab infusion (10 mg/kg intravenously in addition to a first infusion of methylprednisolone) add-on therapy in ten patients with NMO and NMOSD (Clinical trial.gov identifier: NCT01777412). Bevacizumab is a recombinant humanized monoclonal IgG1 antibody that binds to VEGF, and inhibits the interaction of VEGF with its receptors (Flt1 and KDR) at the endothelial cell surface. Bevacizumab (Avastin®) is approved by the EMA and the FDA for several malignant indications. In this study, three patients fully recovered, or even improved, pre-attack neurological function, and no patient required escalation to plasmapheresis. Despite the trial's open-label design, the lack of a control group and the small sample size, bevacizumab appeared to be efficacious and well tolerated. A placebo-controlled trial will be mandatory to determine if the combination therapy is better than the use of high-dose corticosteroids alone [108].

Other cytokines could also be involved. In AQP4-Ab-positive astrocytes, NMO-IgG exposure triggers IL-6 production, therefore highlighting the paracrine function of this cytokine as well. Confirming this hypothesis, when endothelial cells are exposed to IL-6, a decreased barrier function is observed in parallel with an increase in Chemokine ligand 2 (CCL2) and α-chemokine interleukin 8 (CXCL8) expression and an enhanced leukocyte transmigration under flow [109]. Tocilizumab and satralizumab are two mAbs that target IL-6R. The rationale for their use in NMO is the blockade of the stimulation of the production of AQP4-IgG by plasmablasts by this pro-inflammatory cytokine. They are currently being clinically evaluated and seem to show some efficacy in NMO. NMO patients also demonstrate significantly elevated matrix metalloproteinase (MMP)-2, tissue inhibitor of metalloproteinase 1 (TIMP-1), and IL-6 ratio in CSF compared to patients with MS and other neurological disorders [110]. The autocrine secretion of MMP-2/9 by human brain microvascular endothelial cells, induced by humoral factors contained in NMOSD patients' serum, increases blood–CNS barrier permeability, by upregulating Vascular cell adhesion molecule 1 (VCAM-1) protein, and degrades the blood–CNS barrier basement membrane [111]. A better understanding of the pathophysiological mechanisms that lead to the abnormal permeability of the blood–CNS barrier will make it possible to target key players, such as MMPs.

4.2.5 Complement-Targeted Therapy

Following the current dogma, AQP4-IgG production and involvement of CDC and ADCC are both unavoidable steps in initiating the pathological mechanisms of NMO. Complement activation has been observed ex vivo, in mouse spinal cord tissue slices [112] as well as in vivo mouse models of NMO [113]. In such a model, it is mandatory to induce the characteristic histological features characterized by GFAP deposit, inflammatory cell infiltration and demyelination. The development of new complement-targeted therapy in NMO is an important clinical need because the reference C5 inhibitor, eculizumab, is very expensive and is associated with an increased risk of meningococcal infections [114]. Interestingly, the selective inhibition of early steps of the complement pathway preserves lectin activation involved in bacterial killing. Moreover, C1 inhibition prevents the generation of C3a and C3b, which participate in CDC. A neutralizing mAb directed against human C1q protein (with high affinity, 11 nM) prevents AQP4 autoantibody-dependent CDC in CHO cell cultures and NMO lesions in ex vivo spinal cord slice cultures and in mice [115]. Unfortunately, C1q-targeted therapy side effects will differ from those of therapies targeting downstream complement proteins and will not be predictable. C1-esterase inhibitor (C1-inh) could have a potential therapeutic benefit in NMO because it is an anti-inflammatory plasma protein with serine protease inhibition activity and biological activities on the complement pathway [116]. Purified C1-inh from human serum, already used in hereditary angioedema, was administered in ten patients (2000 UI daily of IV Cinryze®) for 3 days at the onset of NMO exacerbation, in addition to standard-of-care treatment (infusions of methylprednisolone) [117]. The aim of this phase 1b, open-label, safety and proof-of-concept trial was to evaluate the effect of C1-inh to minimize complement-mediated damage in acute relapses of NMO. No serious adverse events were recorded. Three out of ten patients were non-responders, two of them requiring escalation to plasmapheresis. In the other seven patients, EDSS scores declined back to baseline or even better. Obviously, this phase 1, open-label, non-controlled trial, with a small number of patients, was not designed for efficacy evaluation and suggests preliminary safety data with C1-inh for patients with NMO. During the same period, using in vitro assays of NMO-IgG-dependent CDC, Verkman's team demonstrated

a minimal inhibition activity of C1-inh at a dose of 2000 units in humans [118]. Therefore, high-dose C1-inh (30-fold greater than that approved to treat hereditary angioedema), injected intravenously, inhibited serum complement activity by < 5% in vivo and did not reduce the pathology in a rat model of NMO [118]. These data suggest that the complement inhibition activity of C1-inh in serum is too low to confer clinical benefit in NMO. Moreover, other challenges, such as CNS penetration, selective inhibition of NMO-inducing complement and the quantity of target proteins, could limit the therapeutic effect of this otherwise attractive strategy.

4.2.6 Polynuclear-Targeted Therapy

In contrast to MS pathogenesis, polynuclear infiltration has been shown to play an important role in NMO-related inflammatory destruction [119]. Neutrophil counts are elevated in CSF in about 60% of untreated patients during relapse, but in only about 20% during remission [120]. Furthermore, administration of granulocyte colony stimulating factor (G-CSF) increased NMO lesions in mice and exacerbated the first episode of NMO in a patient, suggesting a detrimental role for G-CSF in NMO pathogenesis [121]. The proof of concept was established in neutropenic mice in which the intracerebral injection of IgG-NMO and human complement induced less demyelination than in non-neutropenic mice at 24 h and 7 days after treatment [122]. In vivo, NMO lesions were greatly reduced by the intracerebral administration of both neutrophil protease inhibitors sivelestat and cathepsin G inhibitor I or by intraperitoneal injection of sivelestat alone (0.2 µg/g) [122]. Sivelestat is a second-generation neutrophil elastase inhibitor, with a moderate potency ($K_i = 200$ nM) and high selectivity [half maximal inhibitory concentration (IC_{50}) = 5.6 µM porcine pancreatic elastase]. This selective inhibitor of neutrophil elastase is already approved in Japan and Korea for the treatment of acute respiratory distress syndrome. Sivelestat inhibits the degradation of phagocytosed foreign organic molecules within the neutrophil, whereas its extracellular action protects from destruction a variety of extracellular proteins, including elastin, collagen, lung surfactant, and immunoglobulins. In addition to its inhibition of proteolytic activity, neutrophil elastase inhibitor is also known to avoid the production of inflammatory cytokines and to suppress neutrophil-induced capillary permeability and leukocyte kinetics [123, 124]. In an NMO-like mouse model, it was administered daily intraperitoneally, at two doses (0.05 mg and 0.5 mg), from day 6 until day 16 after the transfer of pathogenic Th17 cells [125]. The treatment dose-dependently significantly attenuated the progression of NMO by reducing inflammatory infiltrates in the spinal cord, the optic nerve and the brain. Nevertheless, since 2012, no clinical trial has been performed, maybe because of poor pharmacokinetic properties (low oral bioavailability due to marked intestinal first-pass metabolism and a short half-life requiring continuous infusion to maintain therapeutic effects) and poor clinical efficacy in acute lung injury [126].

Eosinophil infiltration is a prominent feature of NMO lesions, and eosinophils are found in the CSF of NMO patients [120]. Eosinophils can damage cells by releasing toxins from intracellular granules, such as eosinophilic granule major basic protein (MBPe), eosinophil cationic protein (ECP), eosinophil peroxidase and eosinophil-derived neurotoxin. In 2013, Zhang and Verkman demonstrated that eosinophils produce NMO-IgG-dependent pathology by ADCC and CDC mechanisms in ex vivo spinal cord slice cultures [127]. Inhibition of eosinophil degranulation has demonstrated promising results in animal models. Cetirizine, a second-generation antihistaminic drug that selectively antagonizes the H_1 receptor, may exhibit immunoregulatory properties. It was administered orally (10 mg/kg twice daily) in vivo 1 day prior to, and during, the 3-day intracerebral infusion with NMO-IgG and complement in mice. This treatment reduced lesion severity, with fewer eosinophils in lesions. The antihistaminic drug family is quite heterogeneous. Indeed, using three first-generation antihistaminic drugs (chlorphenamine, mepyramine, and hydroxyzine), Zhang and Verkman demonstrated their weak inhibition of eosinophil degranulation in vitro and the absence of any reduction in NMO lesion size in vivo [127]. This is surprising because these compounds are known to cross the blood–CNS barrier, but their activity on other receptors, such as the muscarinic acetylcholine receptor, could perhaps counteract a beneficial effect on the H_1. An off-target effect of cetirizine cannot be excluded, and this encouraged some authors to further explore cetirizine's properties. Recently, an open-label, add-on trial to standard therapy for NMO included 16 patients, who started a treatment with cetirizine, 10 mg daily orally (Clinical trial.gov identifier: NCT02865018). The ARR, initially at 0.4 ± 0.8, decreased after 1 year of cetirizine treatment (0.1 ± 0.24; $p = 0.047$). Despite the open-label design, small sample size and retrospective comparative relapse rate, this pilot study points out the safety and the potential relapse-reducing effect of cetirizine treatment. Moreover, no increase in drowsiness was observed, with this second-generation antihistamine lacking anticholinergic and sedative effects. This trial paves the way for future studies using this alternative agent to prevent the activation and chemotaxis of eosinophils at the beginning of a relapse cascade. Nevertheless, its mechanism of action is unclear and the absence of central adverse side effects raises questions about the origin(s) of its effects in the brain or in the periphery.

4.2.7 Apoptotic Therapy

Bortezomib is a selective inhibitor of the 26S proteasome subunit. The inhibition of the proteasome leads to a decrease in the degradation of regulatory molecules involved in the cell

cycle (IκBα, cyclin E, p53 or p27). The direct consequence in the cell is an increased inhibition of nuclear factor (NF)-κB resulting in an enhanced apoptotic phenomenon. In NMO, bortezomib is hypothesized to induce apoptosis of long-lived plasma cells, and therefore could be an option for patients who are refractory to anti-CD20 therapies. Bortezomib has been tested in one open-label study including five NMOSD AQP4-Ab-positive patients. Among them, four were refractory to a combination of AZA and prednisone and two were refractory to RTX given in the previous 2 years. With the exception of one patient with mild myelitis, the remaining four patients did not relapse 1 year after the 3-month induction phase with bortezomib. This result was associated with an improvement in disability and a reduction of autoimmune activity, reflected by a decrease in serum AQP4-Ab titers and peripheral plasma cell count in all of the patients [128].

5 Remaining Challenges with Current Therapies

There are still some questions regarding the design of the most recent and most robust studies. Many of them propose to investigate add-on therapy in order to avoid placebo-control studies, but this risks being unable to distinguish between a combined effect and a superior effect of the additional drug. Future evaluation in real-life studies will allow us to have an estimate of the ARR under these new therapies and to compare them with older drugs, but this does not constitute level A evidence. Many patients remain disabled even with earlier and more intensive drugs. The major question remains to determine when and with which drug we should treat our patients. Some recent studies argue in favor of intensive treatment, but an escalation should be also proposed. To date, there have been no studies on treatment de-escalation. As patients are frequently young and treated over a long period of time, this strategy should be tested.

6 Development of Biomarkers to Assess Treatment Response/Clinical Outcome

Recent publications have studied the possibility of monitoring the biological effect of RTX by counting circulating B cells. Because $CD19^+$ cells in blood are a good reflection of the global number of circulating B cells, some authors have suggested repeating RTX infusions when $CD19^+$ cells exceed 0.01×10^9/L [64] or when they reach more than 0.1% of the total lymphocyte count [76]. Other authors have suggested that monitoring memory $CD19^+$/$CD27^+$ cells in peripheral blood could optimize the maintenance regimen of RTX, by repeating treatment only when $CD27^+$ cells are above 0.05% of PBMCs [129]. This strategy is supposedly more precise than the monitoring of $CD19^+$ cells, since the risk of reactivation of the disease appears to be specifically correlated with the re-emergence of memory B cells [130]. This strategy could lead to the identification of short-term responder patients who need their RTX infusions to be repeated more frequently than every 6 months in NMOSD [131]. Nevertheless, $CD27^+$ cell monitoring is not an absolute guarantee against the risk of relapse, as demonstrated by Kim et al.: among 100 NMOSD patients, nine patients had relapses (with a combined total of 11 relapses) although memory B cells were below the therapeutic target [49]. Despite these interesting results, this approach seems to have insufficient validity to become a widespread practice at present.

For MMF, the target dosage of 1500–3000 mg/day should be guided by the total lymphocyte count, which should decrease to 1000–1500/μL, following a plasma trough level of 1–2 μg/mL in neuro-immunological indications.

For AZA, a reduced dosage of < 2 mg/kg/day was associated with more relapses, and an increase of the mean corpuscular volume by at least 5 fL from baseline was correlated with fewer relapses [80]. As recommended by specialists involved in organ transplantation, the dose of AZA should be adjusted according to the total lymphocyte count, which should decrease to 600–1000/μL, and according to the mean corpuscular volume, which should increase by about 5% from baseline [80, 132].

Besides the pharmacological action of immunosuppression on lymphocytes, another strategy involves monitoring AQP4-Ab titers. This is a logical approach, based on the effect of treatment on the deleterious antibody itself. A first report underlined the potential interest of such monitoring, demonstrating a correlation between treatment response and antibody titer, especially after RTX treatment [79]. However, this result has not been confirmed elsewhere [133–135]. Thus, we do not recommend monitoring RTX infusion by AQP4-Ab titer in AQP4-Ab-positive NMOSD. This question is also under investigation in MOG-Ab-associated NMOSD, as a link between disease activity and MOG-Ab titer has recently been reported [136].

7 Interesting Targets for Future Therapies

AQP4 is clearly central to the pathophysiology of NMO. Nevertheless, it is unclear whether or not pharmacological therapeutic approaches targeting this protein should reduce or increase its activity. AQP4 activity can only be increased by an augmentation of protein expression or translocation to the plasma membrane, because each expressed channel is assumed to be maximally active in the physiological

situation [137]. On the other hand, it is possible to reduce its activity, but here also, mainly by a change in its expression level; blockers are currently in the first stages of pharmacological optimization. In this section, we will provide an overview of pharmacological and non-pharmacological approaches dedicated to the modulation of AQP4 activity.

7.1 Increasing AQP4 Activity

AQP4 expression can be increased in various situations, such as hypoxia and hypothermia. In rats, a 48-h long hypobaric hypoxia mimicking an altitude of 5000 m above sea level induced a cerebral edema associated with inflammation, demonstrated by an increase of TNF-α and IL-6 and the increased phosphorylation of NF-κB and the Mitogen-activated protein kinase (MAPK) p38, Extracellular signal-regulated kinase (ERK) and c-Jun N-terminal kinase (JNK) [138]. Interestingly, the authors identified that cytokines produced by microglia under hypoxia induce AQP4 overexpression in astrocytes, an overexpression linked to the increase of p38 and NF-κB phosphorylation. These data mean that pharmacological approaches other than TNF-α, but that have in common the augmentation of NF-κB phosphorylation could lead to AQP4 overexpression. Hypothermia can also increase AQP4 abundance in the plasma membrane of astrocytes. In human cortical astrocyte primary cultures, 40-h long culture at 32 °C markedly increased AQP4 localization at the plasma membrane without changing its total protein expression level, meaning that hypothermia favors translocation of the channel to the membrane [139]. This phenomenon depends on transient receptor potential vanilloid 4 (TRPV4) calcium channels, calcium entry into the cell and calmodulin activation because the effect of hypoxia is prevented by a TRPV4 antagonist, a calmodulin inhibitor and by the calcium chelator, ethylenediaminetetraacetic acid (EDTA). On the contrary, in this work, the TRPV4 agonist, GSK1016790A, fully reproduced the effect of hypoxia and, when applied simultaneously, potentiated the effect of hypoxia on AQP4 plasma membrane localization. Considering that this receptor is also expressed at the blood–brain barrier level [140], it would be tempting to test the pharmacological consequences of its activation in NMO animal models and the link with AQP4 activity.

7.2 Inhibiting AQP4 Activity

Ischemic brain injury is a situation that results in brain inflammation and cytotoxic edema. This edema accelerates brain damage, elevates intracranial pressure and ultimately influences long-term outcome. Whereas an ischemic condition should occur in NMO [12, 141], associated with astrocytic edema and an increase in blood–CNS barrier permeability [7–10], decreasing AQP4 activity could be of interest. The role of AQP4 in ischemic edema has been demonstrated in animal models [142] where the overexpression of this protein coincides with early cerebral swelling [143]. Probenecid is an inhibitor of organic anion transporters clinically used as a uricosuric drug. It affects many channels, including pannexin 1, which is described as being involved in cerebral brain ischemia [144]. A recent paper investigated the effects of probenecid on brain lesions induced by focal brain ischemia–reperfusion in mice [145]. Whatever the timeline of administration, this drug limited infarct size and cerebral edema through a reduction of AQP4 expression measured 48 h after the ischemic insult. Messenger RNAs (mRNAs) were not assessed in this work, and it is therefore not possible to know if the protein reduction was due to a reduced expression or to an increased rate of degradation. Nevertheless, neither an increased degradation nor the reduction of AQP4 protein synthesis would appear to be an interesting therapeutic target in this situation because overexpression of microRNA-29b limits brain edema and infarct size in ischemic mice [146]. Other pharmacological approaches could be employed. In rat cultured astrocytes, the AQP4 overexpression induced by an episode of glucose/oxygen deprivation is prevented by progesterone through a PKC-dependent mechanism [147]. It is noteworthy that Protein kinase C (PKC) also activates AQP4 by phosphorylation [148]. Similarly, Goreisan, a Japanese traditional herbal medicine that comprises five herbs, was shown to protect brain from ischemic edema by an inhibition of AQP4 upregulation [149]. This upregulation is at least in part dependent on oxidative stress generated by ischemia. As oxidative stress could be implicated in NMO, an antioxidant approach may be of interest [150]. Resveratrol is a well-known antioxidant that was tested in a model of transient cerebral ischemia in rats [151]. The drug was administered before and two times after the procedure. It reduced infarct size and brain edema in parallel with an augmentation of antioxidant systems and a reduction of AQP4 overexpression. The role of oxidative stress and inflammation in AQP4 expression is now emphasized by the effect of the non-steroidal anti-inflammatory drug piroxicam. With regard to AQP4, this drug is complex because it is suggested to bind to the channel [152], but could also affect the expression of the protein by indirect actions linked to cyclooxygenase inhibition. In a rat model of middle cerebral artery occlusion (1-h occlusion followed by 24-h reperfusion), piroxicam was administered before (30 min) or after (2 h or 4 h) the procedure at doses between 5 and 40 mg/kg [153]. In these conditions, piroxicam reduced infarct size and brain edema and improved cognitive deficit. In the striatum and the cortex, an AQP4 overexpression was induced by ischemia and was blocked by piroxicam. This effect does not argue for a direct effect of the drug on the channel, but rather for a regulation of its expression. Taking into account the diverse pharmacological properties of piroxicam, the mechanism underlying this

effect is unclear. Finally, to conclude this part related to the reduction of ischemia-induced AQP4 overexpression in the brain, some authors evaluated the effect of the statin atorvastatin in a rabbit model of subarachnoid hemorrhage [154]. A high dose of atorvastatin (about 140 times the dose used to reduce cholesterol in humans) reduced AQP4 overexpression (at the protein level) and the associated brain edema and neuron apoptosis. The mechanism by which atorvastatin regulates AQP4 expression is unclear, and lower doses should be investigated.

A reduction of AQP4 overexpression can also be achieved during the natural course of edema following traumatic brain injury. Levetiracetam is an antiepileptic compound that acts by binding to synaptic vesicle proteins. When tested in a rat model of brain trauma, this drug dependently reduced brain water content and AQP4 overexpression at mRNA and protein levels [154]. Unfortunately, this paper did not provide any pharmacological insight to explain the mechanism underlying this regulation. Astaxanthin is a natural carotenoid exhibiting numerous pharmacological properties, including antioxidant and anti-inflammatory effects. Tested in a model of cerebral edema in mice, astaxanthin dose dependently (10, 25, 50 and 100 mg/kg) improved neurological dysfunction, reduced edema and diminished AQP4 overexpression (mRNA and protein) induced by the trauma [155]. This compound also reduced the overexpression of the $Na^+/K^+/2Cl^-$ co-transporter 1 (NKCC1) at the mRNA and protein levels. Interestingly, in this work the diuretic bumetanide, an NKCC1 blocker, prevented AQP4 overexpression. The link between NKCC1 blockade and the regulation of AQP4 expression is unclear, but the regulation of intracellular ion concentration and therefore the cytoplasmic volume could act as a signal to trigger water channel expression.

Regulation of AQP4 expression can also be achieved in situations other than ischemic or traumatic brain edema. A recent paper investigated the effects of the carbonic anhydrase inhibitor diuretic acetazolamide on AQP4 overexpression induced by seizure in a model of temporal epilepsy in Sprague–Dawley rats [156]. At 7 and 28 days after pharmacological induction by lithium chloride/pilocarpine, the authors observed AQP4 overexpression. It was reduced by 35 mg/kg/day acetazolamide with, in parallel, a reduction of both multidrug resistance protein 1 and P-glycoprotein overexpression. This effect leads to the hypothesis that acetazolamide could reduce the risk of developing a pharmacological resistance to antiepileptic drugs. Here also, the mechanism is unclear. The authors hypothesized that acetazolamide is a pharmacological AQP4 inhibitor, but observed a reduction of the expression of the protein. An action on intracellular pH and/or cytoplasmic ion concentration can be speculated. Further studies will clearly be required to investigate all pharmacological pathways affected by diuretics that modulate AQP4 expression in astrocytes.

8 Conclusions

During the past 15 years, our knowledge of NMOSD and its management has developed substantially, moving on from case reports to large cohort and phase III studies. Nobody could have imagined at the beginning of this century that NMOSD would eventually be seen as a clearly separate disease from MS, with disease-specific antibodies (AQP4-Ab and MOG-Ab) and new therapeutic strategies under development, including mAbs. This is the result of a major collaborative effort all over the world, and after the considerable progress on diagnosis, we are now close to class I evidence for a therapeutic effect of several drugs in NMOSD.

Compliance with ethical standards

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Conflict of interest N. Collongues has received honoraria for consulting or presentation from Biogen Idec, Almirall, Novartis, Merck Serono, LFB, Teva Pharma, Sanofi-Genzyme, and Roche, and is a member of the Editorial Board of the *Journal de la Ligue Française contre la Sclérose en plaques*. E. Aylme-Dietrich has no conflict of interest to declare. L. Monassier has no conflict of interest to declare. J. de Seze has received honoraria for consulting or presentation from Biogen Idec, Novartis, Chugai, Merck Serono, LFB, CSL Behring, Teva Pharma, Sanofi-Genzyme, and Roche.

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